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THE RELATION BETWEEN BODY WEIGHT CHANGES AND LIFE DURATION IN MALE RATS*

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The connection between growth and aging has been recognized for some time. In 1564, Cardanus (4) suggested that slow growth was associated with a long life. This hypothesis has received considerable attention in the twentieth century and much evidence has been accumulated in its favor. The literature on this subject has been reviewed in articles by Backman (2), Comfort (5, 6), Heilbrunn (10), Lansing (11, 12), McCay (13), Saxton (19), and Silberberg and Silberberg (22). Perhaps the best known work is that of McCay (14, 16), in which retardation of growth, due to severe caloric restriction, resulted in a significant extension of the life span of rats. However, in a homogeneous group of rats, living on the same diet, Sherman and Campbell (20) could find no difference in the rates of growth between short and long lived animals.

Zucker and associates (27) have formulated an equation which defines optimal growth in rats. It is the purpose of this present study to explore the relationship between changes in body weight and life duration in rats whose growth conforms to the optimum suggested by Zucker.

MATERIALS AND METHODS

Sixty-eight male albino rats, derived from non-inbred Wistar ancestors, were used in this study. The animals were housed in 7 cages, kept in a small air-conditioned room at a temperature of 25 ± 1 C. and relative humidity of 55 ± 10 per cent. Natural light was excluded from the room and artificial fluorescent lighting was kept on for about 8 hours per day.

Animals were fed ad libitum on a diet of rat cubes† which had the following composition: protein 21.7 per cent, fat 2.4 per cent, carbohydrate 55.6 per cent, fiber 4.0 per cent, water 8.3 per cent, and ash 8.0 per cent (consisting of CaO 2.7 per cent, P_2O_5 1.26 per cent, and NaCl 0.7 per cent).

Rats were weighed at weekly or fortnightly intervals to the nearest gram on a Chatillon compression balance from weaning until natural death. Body weights of individual rats at 100, 200, 250, 300, and 400 days of age were obtained by graphical means from these weighings. The growth of these animals closely agreed with the optimum proposed by Zucker and associates (27, 28), except for a short period between 95 and 105 days, when their growth was retarded by an unsatisfactory batch of rat cubes. The maximum weight and the age at which it occurred were obtained directly from the records of body weights.

Zucker's growth parameter (k) was calculated using the formula of Zucker and collaborators (27):

$$k = \frac{\log W_2 - \log W_1}{\frac{1}{t_1} - \frac{1}{t_2}}$$

where k = rate of growth and W_1, W_2 = weights in grams at ages t_1, t_2 days, respectively.

For the calculations of k, over the range of 35 to 80 days, the logarithms of the body weights were plotted against the reciprocals of the ages, and k, the slope, obtained from the graph. The limiting weight (A), or the weight attained if growth continued indefinitely at the

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† Manufactured by Animal Health Products, Chippendale, N. S. W.

TABLE 1. THE RELATION BETWEEN BODY WEIGHT IN GRAMS AND LIFE DURATION IN DAYS.

Group	Life Duration (Range)	No.	Body Weight (Gm.) at				Maximum Weight (Gm.)	Age at Maximum Weight (Days)	Zucker's Limiting Weight (A)
			100 Days	200 Days	300 Days	400 Days			
Very Short Lived	526±13.1 (428–600)	17	247 ±5.9*	340 ±5.8	368 ±6.7	359 ±11.3	384 ±7.4	364 ±18.0	460 ±8.4
Short Lived	655±5.4 (619–700)	17	254 ±6.4	343 ±9.3	376 ±10.4	392 ±11.2	400 ±11.3	425 ±12.4	459 ±12.3
Long Lived	770±12.1 (704–846)	17	256 ±5.8	355 ±8.0	394 ±8.5	414 ±9.2	429 ±9.1	469 ±12.2	492 ±11.8
Very Long Lived	939±16.2 (857–1084)	17	245 ±6.0	343 ±8.3	379 ±9.3	399 ±8.8	413 ±8.9	482 ±14.1	477 ±12.6
Whole Group	726±19.3 (428–1084)	68	251 ±3.0	345 ±3.9	379 ±4.5	391 ±5.5	406 ±5.0	436 ±8.9	473 ±5.8
Correlation Coefficient ± S.E.		68	−0.06 ±0.12	0.05 ±0.12	0.11 ±0.12	0.33 ±0.11	0.51 ±0.09	0.62 ±0.08	0.20 ±0.12
Probability			>.05	>.05	>.05	<.01	<.01	<.01	>.05

* $SE = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$

same rate, was read off the fitted line at infinity on the time scale.

For other values of k the body weights at the various ages were simply substituted in the above formula.

RESULTS

Body Weight and Life Duration.—The body weights of these rats at various ages, together with standard errors, are shown in table 1. In order to emphasize the relation between body weight and life duration, rats have been divided into 4 equal groups in terms of life length. These 4 groups may be arbitrarily described as very short lived (428-600 days), short lived (619-700 days), long lived (704-846 days), and very long lived (857-1084 days), respectively. The change in the mean body weight of these 4 groups with age is shown in figure 1. Smooth curves were obtained in the senescent phase by averaging the weights of both the living and the deceased rats (8, 25).

Analysis of variance was carried out as indicated in table 2. A significant difference was found between the life duration groups ($F = 5.35$, $P < .05$). This effect was due mainly to the significant differences between the body weight at 400 days of the very short lived group and the other 3 groups ($t = 2.82$, 4.70, and

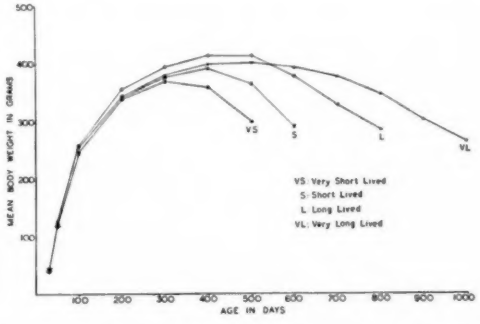

Fig. 1. Change in the mean body weight of 4 life duration groups of rats with age.

TABLE 2. ANALYSIS OF VARIANCE OF BODY WEIGHTS AT 100, 200, 300, AND 400 DAYS.

Source of Variation	Sum of Squares	df	Variance	F	P
Between times	829,335	3	276,445	236.6	<.01
Between groups	26,210	3	8,736	7.5	<.05
Interaction (groups × times)	14,604	9	1,633	1.4	>.05
Error	297,946	256	1,164		
Total	1,168,185	271	4,311		

TABLE 3. THE RELATION BETWEEN GROWTH PARAMETERS OVER DIFFERENT AGE RANGES AND LIFE DURATION.

Group	Life Duration (Range)	No.	k_{35-80}	$k_{100-200}$	$k_{200-300}$	$k_{300-400}$	$\frac{W_{400}-W_{300}}{W_{400}}$
Very Short Lived	526±13.1 (428–600)	17	29.1 ±0.6	27.9 ±1.1	19.7 ±2.1	−11.8 ±5.1	−.029 ±.019
Short Lived	655±5.4 (619–700)	17	28.6 ±0.6	26.0 ±0.7	22.2 ±1.9	19.8 ±4.7	.039 ±.008
Long Lived	770±12.1 (704–846)	17	29.2 ±0.9	28.4 ±1.2	26.6 ±1.7	27.3 ±2.7	.047 ±.006
Very Long Lived	939±16.2 (857–1084	17	27.9 ±0.6	28.2 ±1.0	25.8 ±1.4	28.8 ±2.9	.050 ±.005
Whole Group	726±19.3 (428–1084)	68	28.6 ±0.4	27.6 ±0.5	23.7 ±1.0	23.1 ±2.9	.027 ±.007
Correlation Coefficient ± S.E.		68	−0.12 ±0.12	0.14 ±0.12	0.30 ±0.11	0.50 ±0.09	0.55 ±0.08
Probability			>.05	>.05	<.05	<.01	<.01

3.42, respectively, with $P < .01$ in each case). However there was some contribution from a significant difference at 300 days between the very short lived and the long lived groups ($t = 2.22$, $P < .05$).

Similar information may be gained from a study of the correlation coefficients of the life duration of the whole group of 68 rats on the body weights, as shown in table 1. From these r values it is clear that the early weights were not significantly related to the life duration. Significant correlation was found between life duration and 1) body weight at 400 days, 2) the maximum weight, and 3) the age at which the maximum weight occurred.

Zucker's Growth Parameter (k) and Life Duration.—In table 3, Zucker's growth parameter (k), calculated over various age ranges, has been correlated with life duration. Analysis of variance of this data is shown in table 4. The F values for the variation between groups, between times, and for the groups × times interaction were all significant at the 1 per cent level. Therefore the variation in k with age depends on the life duration group and similarly the variation in k with life duration depended on the age.

Variation in k with age.—In the case of the 2 short lived groups, k decreased with age, but no such change was found in the 2 long lived groups. Using the sampling error variance from table 4, significant differences were found between k_{35-80} and $k_{200-300}$ ($t = 2.06$, $P < .05$) and between $k_{200-300}$ and $k_{300-400}$ ($t = 6.92$, $P < .01$) in the very short lived group. When the standard errors of the individual cells (table 3) were used the difference between $k_{100-200}$ and $k_{200-300}$ in the very short lived group became significant ($t = 3.44$, $P < .01$). In the case of the short lived group, using the standard errors from table 3, significant differences were found between k_{35-80} and $k_{100-200}$ ($t = 2.8$, $P < .01$), and between k_{35-80} and $k_{200-300}$, ($t = 3.2$, $P < .01$).

Variation in k with Life Duration Group.—In the age ranges of 200 to 300 days and 300 to 400 days, k was significantly correlated with the life duration of the whole group of 68 rats (table 3). However the correlation between life duration and the conventional Zucker's growth parameter, based on the early growth (35-80 days), was not significant ($r = -0.12$,

TABLE 4. ANALYSIS OF VARIANCE OF ZUCKER'S GROWTH PARAMETERS.

Source of Variation	Sum of Squares	df	Variance	F	P
Between times	6,590.5	3	2,196.8	12.6	<.01
Between groups	5,980.0	3	1,993.3	11.4	<.01
Interaction (groups × times)	12,986.9	9	1,442.9	8.3	<.01
Error	44,724.9	256	174.7		
Total	70,282.3	271	259.3		

$P > .05$). Scatter diagrams, given in figure 2, illustrate the correlation between k, calculated over various age ranges, and the life duration. In the age range of 300 to 400 days, when the sampling error variance from table 4 was used, significant differences were found in k values between the very short lived group and the other 3 groups ($t = 6.95$, 8.60, and 8.93, respectively, with $P < .01$ in each case). The difference between the short lived and the long lived groups was not significant ($t = 1.65$, $P > .05$).

For k values in the age range of 200 to 300 days, using standard errors from table 3, significant differences were found between the very short lived group and the long lived group ($t = 2.54$, $P < .05$) and between the very short lived and the very long lived groups ($t = 2.42$, $P < .05$).

Relative Weight Increment and Life Duration.—Zucker's equation for the rate of growth is complicated by the use of a logarithmic function. Possibly a growth coefficient could be found which would be more easily calculated and yet yield high correlation with life duration. A relative growth coefficient, the ratio of the change in weight over a given period to the final weight (or initial weight if this is higher) was tested. This simpler measure of growth, when correlated with life duration, gave values for the correlation coefficient (shown in tables 3 and 5) which were slightly

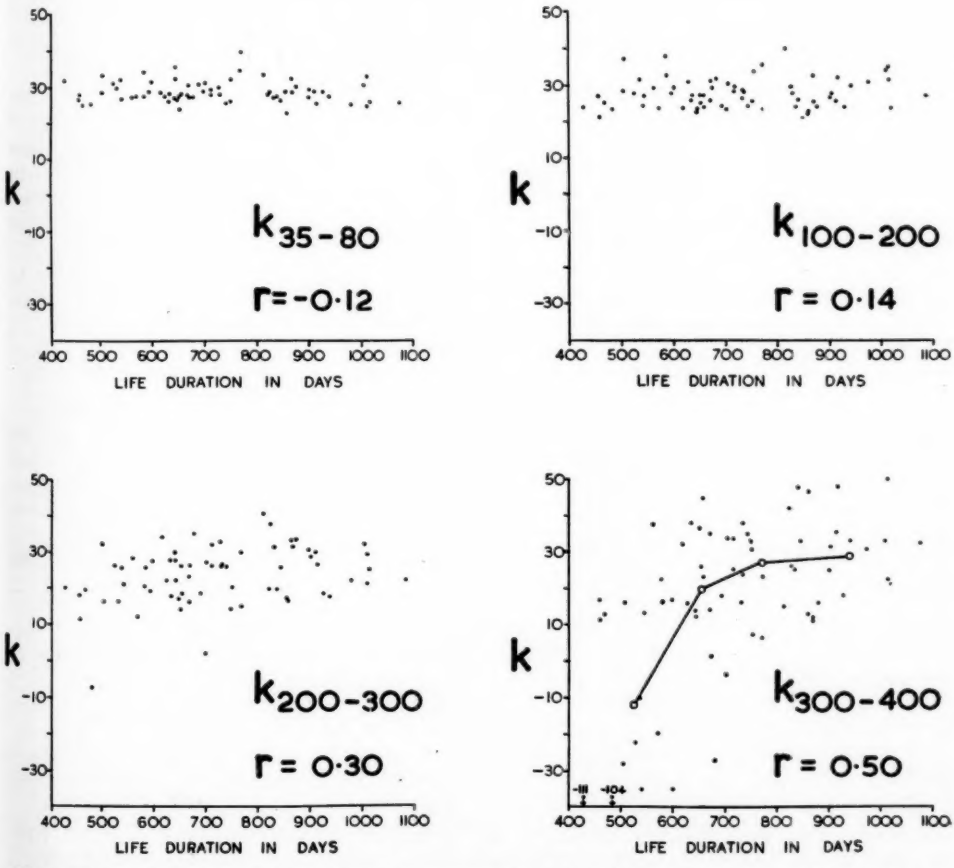

Fig. 2. The correlation between Zucker's growth parameter (k), calculated over 4 age ranges, and life duration. In $k_{300-400}$ the mean k values of each life duration group are joined by straight lines.

TABLE 5. THE RELATION BETWEEN VARIOUS GROWTH PARAMETERS AND LIFE DURATION.

Parameter	Correlation Coefficient ± S.E.	P
$k_{100-400}$	0.48 ± 0.09	<.01
$\frac{W_{400}-W_{100}}{W_{400}}$	0.46 ± 0.10	<.01
$k_{200-400}$	0.49 ± 0.09	<.01
$\frac{W_{400}-W_{200}}{W_{400}}$	0.52 ± 0.09	<.01
$\frac{W_{max}-W_{300}}{W_{max}}$	0.53 ± 0.09	<.01
$\frac{W_{300}}{W_{400}}$	−0.48 ± 0.09	<.01

higher than those obtained with the corresponding k parameter of Zucker. It can be seen from table 3 and 5, that a correlation coefficient of about 0.50 is obtained when the growth parameter involves a function of the body weight at 400 days. There was a slight increase in the magnitude of the correlation coefficient when the growth parameter was based on weight increments from later age ranges.

A still simpler measure of growth, the ratio of body weights at 300 and 400 days, was tested, but gave slightly lower correlation with life duration ($r = 0.48 \pm 0.09$).

DISCUSSION

Disease and the Life Cycle.—The life cycle consists of several overlapping phases of development, loosely defined as growth, maturation, and senescence (22, 24). The stage of senescence is usually characterized by the presence of disease which eventually terminates life (3). In senescence pathologic changes are so common and widespread that it becomes difficult to distinguish them from the changes due to physiologic aging (17). Post mortem examination of elderly people, healthy until their final illness, often reveals the presence of many morbid conditions (3). It would seem that disease and senescence are closely associated phenomena.

In short lived animals the terminal diseases commence at an earlier age than in long lived animals. Restriction of calories in the diet increases the life span of rats by delaying the onset of diseases which precede death (19). In this case it takes a longer time to reach the stage in development when the terminal diseases are due to appear.

In the present study it was found that the faster an animal reached its maximum weight, the sooner it deteriorated and died. The highest correlation coefficient in this work (0.62 ± 0.08) was obtained for the relation between the age at which maximum weight occurred and the life duration (table 1). The very short lived rats reached their maximum weight before other groups of rats because they contracted their terminal illness at an earlier age. A marked loss in body weight occurs in male rats as a result of disease or senescence (8, 15). With the onset of disease in growing rats, growth is suppressed, the maximum weight is attained, and then the body weight falls.

From table 1, it can be seen that the difference between the age at maximum weight and the life duration increased progressively as the life duration increased. Commencing with the very short lived group, the differences were 162, 230, 301, and 457 days, respectively, which were all significant at the 1 per cent level. Note that the delay of 162 days between the maximum weight and death, in the case of the very short lived group, is equivalent to 16.2 years of human life. Thus even in the very short lived animals chronic disease may have been present. Autopsy revealed a 94 per cent incidence of lung disease in this very short lived group as against 94, 76, and 65 per cent, respectively in the longer lived groups. According to Saxton (19) chronic pneumonia is first detectable in rats at 250 days.

Body Size and Life Duration.—It is known that in mammals the larger forms live longer than the smaller, probably because of their lower metabolic rate per unit weight (10). This relationship is also approximately true for members of the same species and sex. For male rats of this non-inbred Wistar strain, the life duration was proportional to the body weight maximum. Our value for the correlation coefficient (0.51 ± 0.09) agrees well with that previously obtained by McCay and associates (15) on male rats of the Yale strain (0.482 ± 0.05). On the other hand, the limiting body weight, the A parameter of Zucker and associates (27) based on the initial rate of growth, was not significantly correlated with life duration ($r = 0.20 \pm 0.12$, $P > 0.05$). Since A represents the weight that a rat would ulti-

mately attain if its growth was not cut short by disease or senescence, it is relatively independent of the aging process. This is not so for the maximum weight. A rat, in which the terminal disease starts early, has its growth process interrupted prematurely. Consequently its maximum weight is approximately equal to the body weight at the time of growth suppression by illness. The maximum body weight is thus more intimately connected with disease and senescence than is the limiting weight, A.

The longest lived rats were generally those of moderately large body size. The heaviest rats were, however, at a slight disadvantage with respect to longevity, since most of them died during the period of 704 to 846 days (third life duration group). This result could be due to the well known effect of overweight in diminishing life expectancy (1, 7). McCay and associates (15) drew attention to the fact that the maximum weight depends not only on body size but also on the degree of fat deposition.

Rats with low body weight maxima were usually those animals which developed their terminal illness early in life. Nevertheless a few rats of small body size did have a long life. Two brothers with maximum weights of 347 and 348 Gm. lived for 958 and 846 days, respectively. However, other members of this litter gave quite different results (364 Gm., 457 days; 390 Gm., 644 days; 409 Gm., 655 days).

It is important to realize that the sex differences in body size and life duration are not consistent with this direct relationship between maximum body weight and life expectancy. Females in many species are of smaller body size than males and yet are longer lived. Also growth retarded rats are smaller than normal rats but live longer (14, 16).

By the time the maximum weight is reached degeneration has already begun. Correlation between life duration and body weight at an earlier age than the time of maximum weight would be of greater value in forecasting life expectancy. At 100 and 200 days the 4 life duration groups were indistinguishable. At 300 days a statistically significant difference was obtained between the very short lived group and the long lived group, but this was only of limited value in predicting life expectancy since the very long lived and very short lived groups were not significantly different. From the values of the body weight at 400 days it was possible to separate the very short lived rats from the other 3 groups. However, the 3 longer lived groups could not be separated, at this time, on the basis of body weight.

Growth and Life Duration.—The relation between growth and life duration is dependent on a third variable, namely diet. There is now an abundance of evidence to support the theory that retarded growth, produced by a diminished intake of calories, is followed by prolongation of life (13, 23). On the other hand Sherman and Campbell (21) showed that the addition of certain nutrients to an already adequate diet leads to both an improvement in growth and an increase in the length of life. In this present work the composition of the diet was maintained constant.

From table 3 it can be seen that the initial rate of growth, as measured by Zucker's k parameter, was not significantly correlated with life expectancy. These results confirm the studies of Sherman and Campbell (20), who found no significant difference between the growth rates, in the age range of 5 to 8 weeks, for short and long lived rats.

Also in agreement with our findings is the observation of Zucker and associates (27, 28) that all rats of a given strain, when fed a diet producing optimal growth, have approximately the same rate of growth, k. Now in published longevity studies one striking feature is the great variation in the life duration of rats (9, 14, 15, 20, 26). Consequently if Zucker's k can be taken as constant and the life duration is highly variable, then the life duration is independent of k, the rate of growth.

This leads to the conclusion that Zucker's growth parameter, based on the early growth, is of little value in predicting the life expectancy of rats. Indeed it is the deviation from Zucker's log reciprocal line that is of importance. In the age range of 200 to 300 days both the very short lived and the short lived groups of rats deviated significantly from the initial k values. These 2 groups could be separated in the next age range of 300 to 400 days, when their k values were significantly different at the 1 per cent level. However, at no age range up to 400 days was it possible to separate the long lived and the very long lived groups on the basis of their k values. In effect, rats could be divided into 3 life expectancy classes (very short lived, short lived, and combined long and very long lived) from an analysis of their growth in the age range of 200 to 400 days.

A similar investigation was carried out by Robertson and Ray (18) who found that short lived mice had the slowest rate of growth. In our series the slowest rates of growth occurred in the 2 short lived groups, in the age range of 200 to 400 days. These two observations would seem to contradict the work of McCay (14, 16), which clearly demonstrated that a reduction in the rate of growth led to an increase in the life expectancy.

The reason for the difference presumably lies in the cause of the reduced growth. In the normal life cycle of the male rat the body weight rises rapidly during the period of growth, increases slowly during maturity, and then declines in senescence (8). In our case, the very short lived rats had a reduced rate of growth because of disease or senescence, and consequently had a poor prognosis.

On the other hand, the reduction in the rate of growth in McCay's animals was due to caloric starvation, which slowed the process of aging. Even after 900 days of retardation, growth could be resumed by feeding a diet adequate in calories (13). This suggests that after 900 days of retardation these rats were still living in the phase of growth.

While the present study throws no light on the mechanism of aging, it is of value in sorting rats into life expectancy groups. Results from this work may be used in matching the physiologic histories of body weight in rats, before applying the method of random selection to grouping. In this way animal groups may be rendered more homogeneous for longevity studies. The great variation in life duration that occurs even with inbred strains of rats (26) indicates the need for better grouping procedures. The relation between body weight changes and life duration is most useful when applied to the grouping of middle-aged male rats.

SUMMARY

In the case of male rats living on a diet which produced optimal growth, the life duration was independent of the initial rate of growth, as measured by Zucker's growth parameter, k.

In short lived rats the rate of growth decreased significantly in the age range of 200 to 400 days. In long lived rats there was no corresponding change in the rate of growth in this period.

Inherent body size, as measured by Zucker's limiting weight parameter, A, was not significantly correlated with the length of life. This was in distinct contrast to the high correlation between the observed maximum weight and life duration.

The highest correlation coefficient was found for the relation between life duration and the age at which the maximum weight occurred.

The application of these results to the forecast of life expectancy and to the grouping of rats in longevity studies was suggested.

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AN AGE INVOLUTION IN THE SMALL INTESTINE OF THE MOUSE

With a Description of the Fundamental Process of Lymphoepithelial Metamorphosis in Intestinal Mucosa*

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There is evidence in the literature to indicate the occurrence of changes in the alimentary tract associated with the aging process. A condition known as atrophic gastritis is present in over 80 per cent of normal persons from the fifth decade on, according to some authors (25, 27, 46). This condition involves a decrease in the thickness of the mucosa, an increase in infiltration by leucocytes and in aggregates of lymphoid tissue, and a dedifferentiation of fundic glands toward the pyloric type.

The frequency of occurrence of benign polyps of the gastric mucosa increases with age (26, 47).

In an early study Goodpasture (24) found multiple polyps in the pyloric antrum in 7 out of 50 senile dogs.

It is very difficult to obtain reliable data in relation to the possible age changes in the small intestine in man, chiefly because the degree of inanition preceding death can lead to marked changes through edema of the mucosa and atrophy and sloughing of the epithelium. It is generally believed, however, that both the mucosa and the musculature of this organ undergo atrophic changes in old age (28).

For the colon, too, the statement has been made that atrophy occurs in old age. This seems probable when the definite increase of diverticulosis is considered.

For one part of the intestinal tract, a particular kind of change seems to occur in a large number of older individuals. The vermiform appendix showed obliteration of the lumen in over half of the individuals over 50 years of age in a series studied by Ribbert (41). This was in the absence of evidence of current or previous inflammation and therefore probably indicates a true involutionary process on the basis of proliferation of some relatively inert type of tissue.

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The paucity of data in the literature on histologic changes with age in the alimentary tract and the availability of material from a number of relatively healthy senile mice in our colony of C57 Black animals made it seem desirable to study the intestine in a series of these animals. As an incidental observation in an earlier work (4) a change in type of connective tissue had been seen in 3 mice of this strain, 1 of middle-age and 2 in the senile category. What the significance of this change might be and how closely associated with the aging process it is could not be studied further at that time due to lack of availability of material.

MATERIALS AND METHODS

For the present study we have used 8 young adult animals (128-248 days, 6 female, 2 male); 1 animal of late middle-age (573 day female), and 9 definitely senile mice (713-818 days of age, 3 female and 6 male).

The animals were anesthetized by injection with veterinary nembutal (0.2 cc. of a solution of 60 mg. per cc.) and the intestine removed while the heart was still beating. We should not have to consider, then, the factor of postmortem change. There is some variation in the degree of adherence of the epithelium to the tunica propria of the villi in different animals, which may be because of different states of digestion at the time of sacrifice or other causes.

A segment of small intestine 4 or 5 mm. in length was fixed in Zenker's fluid, dehydrated, cleared, infiltrated, embedded in paraffin, cut at 6 μ, and stained with Ehrlich's hematoxylin and eosin. This piece corresponds to the upper portion of the jejunum in man.

The sections were surveyed with the low power (100 diameters) for the general appearance of the layers of the wall of the intestine, the relative amounts of fibrous connective tissue, and any other features of note. Then these features were studied with higher magnifications, including oil immersion.

TABLE 1. CHARACTERISTICS OF LYMPHOCYTES AND MITOTIC FIGURES IN THE CRYPTS OF LIEBERKÜHN IN THE SMALL INTESTINES OF 18 C57 BLACK MICE IN FIELDS INVOLVING AN AVERAGE OF 1296 EPITHELIAL CELLS PER ANIMAL, OR A TOTAL COUNT OF 23,328 EPITHELIAL CELLS.

Animal Identification	Age in Days, Sex	Degenerating Lymphocytes			Unaltered Lymphocytes & Lymphocytes in Direct Transformation			Mitotic Figures			
		Apical	Level	Basal	Apical	Level	Basal	Prophase Metaphase	Anaphase (Aberrant)	Anaphase (Normal)	Telophase
1121	128F	2	0	3	18	27	28	15	0	3	4
1122	129M	7	1	3	10	9	21	23	9	8	9
1124	129F	8	3	1	11	10	15	32	6	3	11
1125	130F	9	0	2	14	14	16	21	5	8	11
1126	131F	3	0	1	16	11	20	34	7	2	6
1127	134F	12	0	1	10	14	20	17	13	3	10
1128	134F	11	2	0	17	13	17	16	9	3	12
2261	248M	4	0	1	12	14	15	35	7	8	4
5505	573F	20	1	7	7	10	2	29	9	5	10
5631	713F	13	1	2	4	5	8	39	5	7	16
5632	716M	21	6	3	3	3	14	36	9	0	5
5634	716M	15	1	1	6	4	2	36	12	5	18
5512	719F	12	0	2	1	5	7	43	6	6	18
5517	733M	21	0	3	8	3	3	44	2	2	14
5521	733F	8	0	3	6	11	4	40	5	6	17
5688	784M	5	2	3	6	5	6	42	8	8	15
5686	788M	16	2	3	1	6	5	39	7	4	17
5646	818M	12	2	1	13	17	6	26	9	3	11

A critical study was made under the oil immersion lens of the cells and cellular relationship in the crypts and on the villi. In the crypts, fields involving an average of 1296 epithelial cells per animal were studied, the total being thus 23,328 epithelial cells. Actually, the area used was simply such as needed to include observation on 100 lymphocytes and mitotic figures.

In table 1 the lymphocytes present in the crypts are characterized according to their condition as degenerating or unaltered lymphocytes and lymphocytes in direct transformation. The lymphocytes are further characterized by their position as basal, meaning below the epithelial nucleus, level, meaning at the same level as the epithelial nucleus, or apical, meaning above it.

On the villi, 20 oil-immersion fields were studied in each animal. Here the position, shape, and condition of the lymphocytes were noted. Mitotic figures are not present on the villi.

The quantitative data on cellular relationships presented in this paper concern the crypts of Lieberkühn. Since special features exist in relation to the villi, we have reserved their detailed consideration for a future paper.

OBSERVATIONS

In all of the young animals the typical histologic picture of the wall of the small intestine is seen. There is one difference from the wall of the intestine in man in that the mouse lacks a submucosa. The tissue between the epithelium and the circular muscle layer may all be designated, then, as tunica propria of the mucosa. In the young animals the crypts lie close together (fig. 1) and the tunica propria is highly cellular, with an abundance of lymphocytes and plasma cells and occasional polymorphonuclear leucocytes.

In all of the 10 older animals a marked change has occurred. There has appeared a type of fibrous connective tissue in the tunica propria between the crypts which is almost devoid of cells (figs. 2 and 3). The cores of the villi remain as a highly cellular tissue. In the region of the crypts, however, the replacement often is very extensive and no features other than blood-vessels may be seen amid the peculiar connective tissue which has developed about the epithelial tubes. This tissue itself occurs in coarse blocks, ovoid or rectangular in shape in these cross-sections, each block some 20-40 μ in diameter. In longitudinal section these structures appear as coarse fibers.

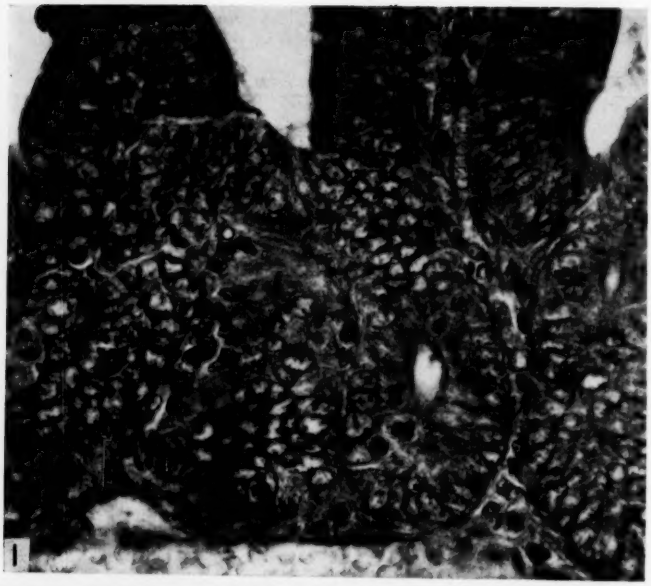


Fig. 1. Mucosa of the small intestine of a 129-day male mouse. The crypts lie very close to one another and the tunica propria is highly cellular. X570.

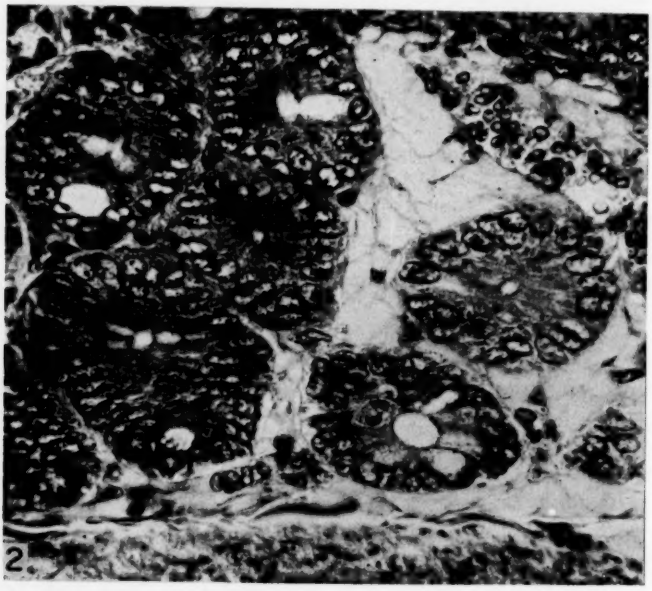


Fig. 2. Mucosa of the small intestine of a 788-day male mouse. The crypts in some places are separated widely from each other. The intervening space is occupied by coarse, lightly eosinophilic fibers, seen here in cross-section. X570.

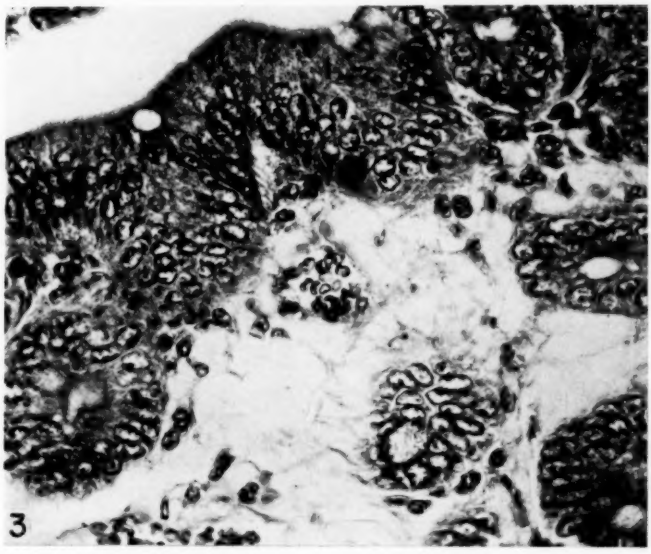


Fig. 3. Mucosa of the small intestine of a 788-day male mouse. A large amount of fibrous connective tissue, with very few cells, is seen. A portion of the surface epithelium between the villi is visible. X570.

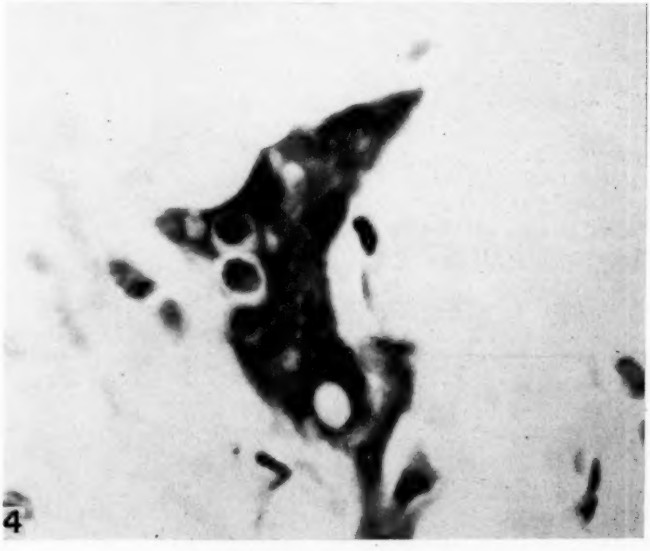
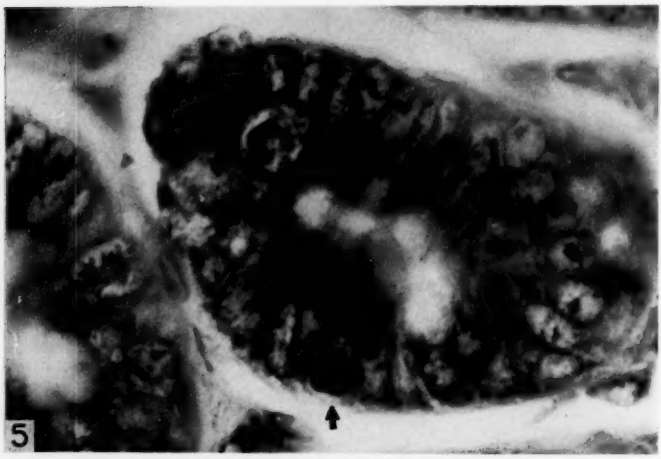


Fig. 4. Peculiar spicule-like basophilic body found in the connective tissue of the tunica propria in a male mouse of 818 days. Cells within such bodies resemble lymphocytes and plasma-cells. X1352.



Magnification of figures 5 through 7 is X1352. In these figures the ages are given only for specimen identification. There is no indication of any essential difference in the process of metamorphosis in the two animals.

Fig. 5. A lymphocyte, denoted by arrow, which has just entered the epithelium of a crypt. It seems to have displaced an epithelial nucleus apically. From a 573-day female mouse.

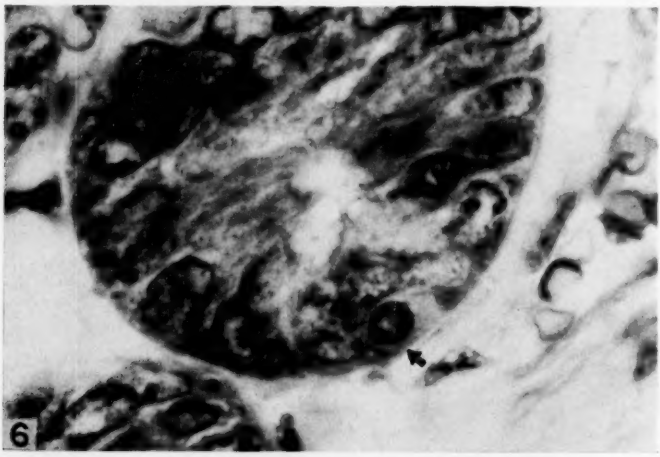


Fig. 6. A transforming lymphocyte (arrow), of which the cell-body has enlarged and the nucleus is becoming more vesicular. Note again the displaced epithelial nucleus. From a 784-day male mouse.

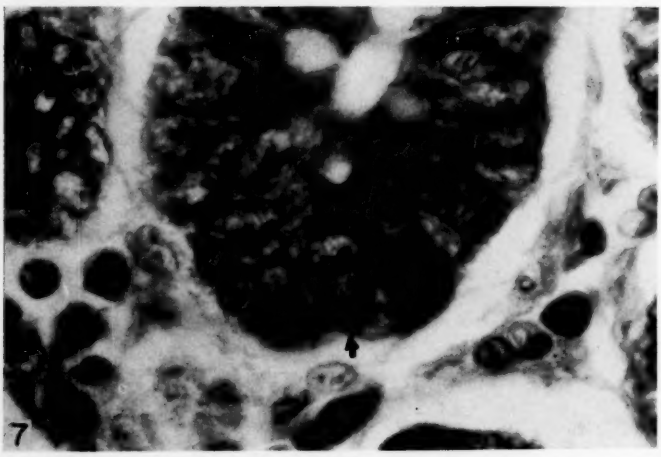


Fig. 7. An almost completely transformed lymphocyte (arrow). The cytoplasm stains similarly to that of epithelial cells and the nucleus is more epithelioid than lymphocytoid. Note the deformed epithelial nucleus above this cell. From a 573-day female mouse.

In a few areas in the senile animals a basophilia of the connective tissue is seen. In one animal, our oldest specimen, this is a conspicuous phenomenon. Masses of considerable size and of irregular shape, often with sharp angles (fig. 4) are scattered in the tunica propria. They usually contain cells, often within clear spaces. All in all, these basophilic masses resemble rather strongly spicules of cartilage. The cells seem to be of more than one type, their nuclei resembling in some cases those of lymphocytes, in others those of plasma cells.

The histochemical test for calcium, by the von Kossa impregnation method, has proved negative. Thus the nature of these basophilic structures is not clear.

In making a detailed study of the epithelium of the crypts and of the villi for possible points of comparison or contrast between the young and old specimens, we have been brought again to the consideration of the fundamental question of lymphoepithelial metamorphosis, a subject which we considered in several earlier papers (2, 6, 7, 8).

We find abundant evidence of a direct metamorphosis of lymphocytes to epithelial cells all along the villi. The lymphoctyes are found primarily in two locations: 1) basal to the row of epithelial nuclei and 2) at the level of this row of nuclei. The apical position is rare on the villi, although very common in the crypts of Lieberkühn.

The transformation of lymphocytes to epithelial cells on the villi is indicated by their taking up a position in which their nuclei are at the same level as those of the epithelial nuclei; by the intercalation of their cytoplasmic bodies snugly between those of the epithelial cells; by the enlargement of these bodies; and by the internal changes in the nuclei toward the lighter epithelial type. This transformation involves the change from an amoeboid flowing cell to a columnar, stable one. In many instances the extension of the cytoplasm of the lymphocyte to the lumen is initially in the form of a long, clear, more or less pointed process.

In the crypts of Lieberkühn the aspect of migration and transformation of lymphocytes is a more complicated one than on the villi. Here too there is abundant evidence of a process of direct transformation (figs. 5 though 7) but numerous instances of degenerative change and of mitotic division also are seen.

The numbers of lymphocytes according to position in relation to the epithelial nucleus and to state of lymphocytic nucleus and cytoplasm and the number of cells in the different stages of mitosis are given in table 1. While individual variations in numbers of lymphocytes are great among these animals, the numbers of unaltered lymphocytes and lymphocytes in direct transformation is very high in the young as compared to the older animals, with the exception of specimen 5646, the oldest animal of all. This animal presented a special case, with large numbers of unaltered lymphocytes, amounting to a picture of infiltration in the crypts.

The analysis of the location and condition of the lymphocytes in the crypts contributes greatly, we feel, toward explaining their role here.

We have suspected for a long time (2) that the description of the great amount of mitosis in the crypts of Lieberkühn as representing an uncomplicated multiplication of the intestinal epithelium is an oversimplification. The early presentation of the wander-theory (12, 13) according to which the only real replacement of epithelial cells occurs in the crypts and the newly formed cells push the older ones up onto the villi where they eventually are sloughed off or desquamated was received favorably by many authors. Some recent workers (35) attempting to work out quantitative data concerning the time required for each complete renewal of the intestinal epithelium in the rat, have used this theory as a basis and have felt that new evidence in favor of it was afforded by their own observations.

When we subject the crypts of Lieberkühn to very close scrutiny and pay attention not only to the epithelial cells but also to the lymphocytes and to the relationships between the lymphocytes and the epithelial cells, we see many features which not only are unexplained by the wander-theory but which seem to indicate a wholly different type of process.

The behavior of the lymphocytes cannot be described as a simple migration into the crypt with subsequent discharge into the lumen. There is a marked tendency for the individual lymphocytes to enter the epithelial cells. Clearly intracellular lymphocytes are present in large numbers in the apical portion of epithelial cells (table 1 and fig. 9). Numerous lymphocytes are seen intercalated between the bases of two epithelial cells, while some are farther apically in

the intercellular position, at about the level of the nuclei of the epithelial cells. It would seem that the more common course of events for such lymphocytes as are to become intracellular in position is for them to migrate between the cells to a site beyond the epithelial nuclei, then to enter into the epithelial cytoplasm. Since none of the lymphocytes outside of epithelial cells show any sign of degeneration, the entry may be an active process, but this remains to be demonstrated. There are, it is true, some degenerating lymphocytes in a basal position which seem to be intracellular and to have entered the epithelial cells directly from the tunica propria.

The outstanding feature about the lymphocytes in the intracellular position is that practically all of them, whether basal or apical, are undergoing a marked change. The changes which the lymphocytes are undergoing are complicated. In some cases the nucleus is tremendously increased in size (fig. 8). In others it is reduced to a tiny sphere in a large vacuole. In practically all cases, however, a process which we may call chromatin condensation is occurring (figs. 8 and 9). The chromatin becomes condensed into a few densely basophilic masses or sometimes into a single mass, leaving the remainder of the interior of the lymphocyte nucleus as a homogeneous eosinophilic sphere. The condensed chromatin usually is peripherally located. The eventual outcome of this change in many cases seems to be degeneration of the lymphocyte. Cases of frank breakdown or karyorrhexis are seen, while the reduction in size of many nuclei, within a clear vacuole, indicates karyolysis. Actually, extrusion of the degenerating lymphocyte is a rare phenomenon, the products of degeneration seeming to be destined to dissolve into the epithelial cytoplasm. Undoubted lymphocytes in process of degeneration within epithelial cells, then, are conspicuous in the crypts of normal animals at all ages studied and it seems surprising that they have not been described by other workers. It may be, however, that they have been confused with the mitotic figures which are so abundant in this region.

Mitotic figures, prophase through telophase, generally are from twice to several times as numerous as are the degenerating lymphocytes. It is these mitotic figures which have been assumed to be those of epithelial cells. Here we wish simply to describe the mitotic process and its variations as seen in the crypts. The probable significance of the special features of mitosis will be considered.

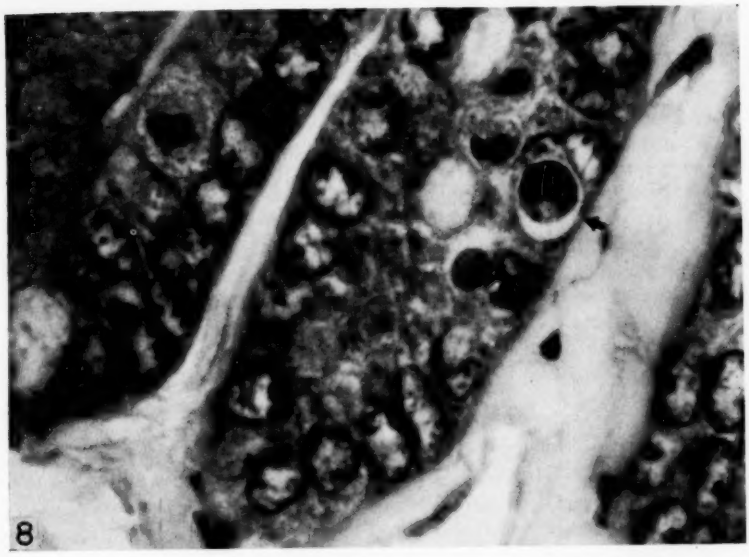
It must be said, however, as a matter of objective observation, that there is a very considerable resemblance between some of the degenerating lymphocyte nuclei and some of the mitotic figures (fig. 9). This is particularly true of the prophase. The prophase presents a nucleus with the chromatin in the form of a thick ring. It may well be said to be condensed chromatin although the degree of condensation is not as great as that in the frankly degenerating lymphocyte nuclei. The central portion of the nucleus remains relatively lightly stained (fig. 9).

It is true that nuclei presenting more of the classical picture of a prophase, with the coiled thread or spireme, can be identified from time to time. Very often these nuclei can be shown to be members of a pair in process of reconstitution, in other words, actually telophase figures. On the other hand, some of these nuclei may be actual epithelial prophases. The great majority of nuclei which are in the initial stages of mitotic division in the crypts, however, show the ring-shaped appearance. There seem to be no transitional stages between a vesicular, epithelial-type nucleus containing a spireme and the relatively dense ring type of prophase.

The metaphase is not a stage easy to identify with certainty. In some figures the masses of chromosomes lined up on the equatorial plate can be seen in profile. In others they can be seen peripherally on the spindle in polar view. Generally, however, it is difficult or impossible to separate the prophases and metaphases and we have believed it better to group the two together. The anaphases and telophases are readily identifiable as such.

It will be seen from table 1 that the combined group of prophases and metaphases represents actually the great majority of the figures. This is not surprising when one considers the longer duration of the prophase as compared to the other stages of mitosis.

The anaphase figures in the crypts of Lieberkühn present a considerable variety. While many of them are similar to the typical picture of this stage, with well-formed spindle and with the two discrete groups of chromosomes, proceeding toward opposite poles, others show varying degrees of what seems to be failure of the chromatin to become individualized. There



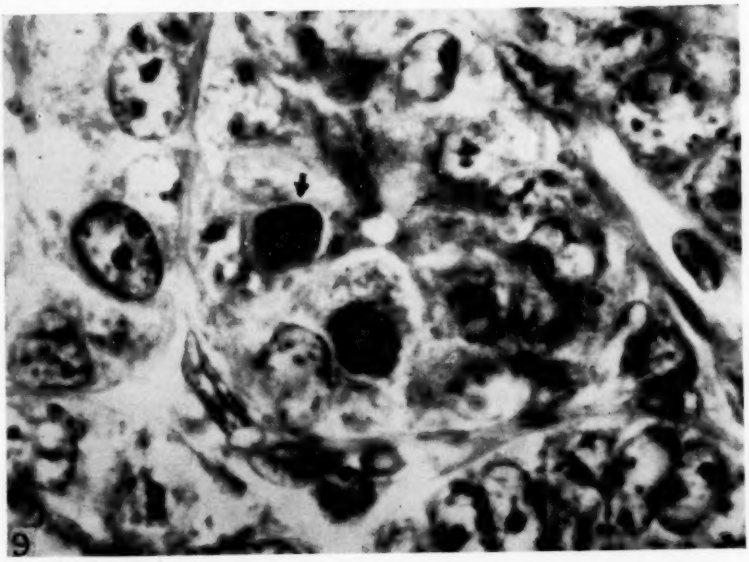


Fig. 8. Crypts of Lieberkühn of a 733-day male mouse. A very large lymphocyte nucleus in process of degeneration is seen above (arrow), while in the epithelial cell below it is a completely eosinophilic lymphocyte nucleus, the condensed chromatin having disappeared. The crypt to the left shows a cell, which we interpret as a lymphocyte, in prophase. X1995.

Fig. 9. Crypt of Lieberkühn of a 721-day male mouse. To the left and above (arrow) is seen a frankly degenerating lymphocyte within the apical portion of an epithelial cell. Below it is one of the ring-shaped prophases. We believe that the first figure represents a lymphocyte which has been unsuccessful in initiating a differentiating mitosis, while the second represents one which probably will carry through to produce two daughter epithelial cells. This specimen is Regaud fixed, stained with acid fuchsin. X1995.

seems to be a tendency for the condensed chromatin to remain so, to clump, cling, and adhere.

The frequency of chromatin bridges, often robust and extending for considerable distances, is surprising for normal material. It will be seen from table 1 that the number of aberrant anaphases often is as great as that of normal anaphases and in some cases, as in specimen 5686, they are many times as numerous. Although there are many anaphases which are clearly aberrant, there seem to be few if any which would be unlikely to proceed to telophase. In other words, in contrast to some figures in the prophase-metaphase group, there is no indication in the anaphase of a tendency to a breakup of the nuclear material with eventual death.

The telophases have been identified as pairs of definitely reconstituting nuclei (two nuclei = one telophase). An early telophase shows two relatively small dense nuclei of ovoid or often of somewhat kidney-shaped contour with the hila of the two nuclei facing each other. Later telophases show larger, brighter nuclei often with a spireme and a clear nuclear sap in which nucleoli are appearing. The telophase nuclei are clearly in process of becoming more like the nuclei of the epithelial cells. No aberrant features have been noted by us in this last stage of mitosis.

Single nuclei which could be either in a later telophase or in a prophase of an epithelial cell are seen. In many cases their form seems to indicate a telophase rather than a prophase even though the sister nucleus is not identifiable in the section. We have not attempted extensive use of thin serial sections to resolve this matter. Epithelial nuclei which by form and internal structure could be in prophase are extremely rare in our experience.

DISCUSSION

One of the more prominent generalizations which have been made concerning the process of senescence is that there is a tendency to fibrosis in many organs, a replacement of the parenchyma of an organ and of the originally delicate stroma by a coarse, heavily fibrous tissue. In a recent article (3) we have pointed out that a study of organs of laboratory animals does not substantiate this view for many important organs (liver, kidney) but that in others (salivary glands) a great deal of replacement of parenchyma by adipose tissue occurs while in still others (thyroid gland) a really extensive fibrosis occurs. It seems from the results of the present study that the tunica propria of the small intestine is another site in which fibrosis is a prominent feature in old age. The occurrence of this process in all of the senile specimens in the present series of animals, which had been kept under optimum conditions in our laboratory for approximately the second year of their lives, seems to us to obviate infection or other pathologic condition as a probable cause of this change.

We have considered the possibility that the almost hyaline type of connective tissue might represent a deposit of amyloid material. Such deposits have been described by Dunn (19) as appearing spontaneously in some strains of inbred mice (A and C_3H) in a number of localities, including the interstitial tissue of Brunner's glands, portions of the submucosa of the stomach, intestinal villi (location not mentioned), and the colon, as well as in the heart, veins, gonads, adrenals, and, in particular, the kidney (48). The amyloid deposit has an effect similar to that of our fibrotic change in separating the parenchymal elements widely from each other and low power figures show a considerable resemblance. Also, the animals used in the study by Dunn "had arrived at an advanced age." However, the interstitial material with which we have been dealing, while appearing rather homogeneous under low power and showing very few cellular elements, is of a coarsely fibrous nature. Therefore, the relation between it and spontaneous amyloid deposits in mice of advanced age, if any relation exists, remains problematical.

The peculiar basophilic bodies found in one of our animals, the 818 day male, are of interest in being found in the specimen of most advanced age and in seeming to represent a still further change in the already altered interstitial tissue. Morphologically, the basophilic masses resemble pieces of cartilage and the manner of entrapment of cells within lacunae in the matrix recalls the happenings in the formation of such skeletal tissue.

The abundance of lymphocytes in intestinal mucosa and their movement into the epithelium under normal conditions has long been recognized (40). The role of the lymphocytes migrating into the intestinal epithelium is one of immense importance for general concepts of cellular relationships and potentialities. In the

present study, with observation and comparison of sections of intestine from a number of relatively normal animals of known age and pedigree, there has been an excellent opportunity to review the relationship of lymphocytes to epithelial cells.

Our own ideas concerning the role of the lymphocyte have developed over a period of years and as a result of a number of individual studies by our colleagues and ourselves. Our first study of lymphocytes in intestinal epithelium in the mouse (4) led us to conclude that these cells actually enter into the epithelial cells and that it is they which undergo mitosis. These conclusions were supported by further studies on human material (9) and by further studies on the mouse with greater attention to cytologic detail (10). Observation of the course of mitosis led us to the conclusion (2) that differentiating mitosis occurs in the crypts since lymphocytes enter upon mitosis, epithelial cells result from it. Many details of evidence, however, were lacking and at that time we were not yet aware of a direct process of metamorphosis of lymphocyte to epithelial cell without mitosis.

A study of the normal epidermis of the rat and of man, projected primarily to ascertain changes with age, led to the finding of a constant occurrence of lymphocytes in the basal layer and of evidence there of a direct metamorphosis of lymphocytes through clear cells to epithelial cells (6). Two quantitative studies on lymphocytes in epidermis of the rat at various ages (7, 8) served to confirm the occurrence of such a direct metamorphosis.

An examination of the manner of replacement of the intestinal epithelium in urodele amphibians (39) showed a process of migration of cells from the cell-nests into the surface layer of epithelium and a differentiation there to columnar epithelial cells. Further, the population of the cell nests appeared to be a mixed one, with mesodermal elements (lymphocytes) among the larger cells and seeming to differentiate into them.

All these findings over many years encouraged us to the present detailed observations on the intestinal epithelium of the mouse in this series of animals having a wide range of age. In many organs, such as liver, kidney, and other glands, the parenchyma seems to be rather well set off from the cells which are circulating in the blood or wandering through the interstitial tissues. In fact, as we have pointed out (3) for several organs the breakdown of the barrier between parenchyma and blood or interstitial tissue in old age seems to be related to degenerative changes in the parenchyma of the organs involved. Sommers (43) believes that in some cases the most likely function which lymphocytic aggregations may serve is "to act upon and alter chemically the soluble autologous proteins that leak from unprotected cells, in a way analogous to lymphocytic effects upon the mild irritants or toxic materials in lymph passing through lymph nodes."

In any event, we know that many surface epithelia are normally the site of lymphocyte migration. In this regard, it may be important to note the emphasis which has been placed by some authors (32) upon a biologic classification of tissues, based on such traits as duration of life of cells, reproductive and regenerative properties, and character of growth. Both in relation to problems of aging and of neoplastic growth, this classification often seems more useful than does that of the 4 groups, epithelium, connective tissue, muscle, and nervous tissue, which is used commonly.

According to the biologic classification, there are 3 groups of cells: perennial elements (such as nerve cells and striated muscle); stable elements (cells of liver, pancreas, kidney, smooth muscle cells, neuroglia); and labile, unstable elements (blood cells, germ-cells, and surface epithelia). In this classification, then, the surface epithelia are found in a different category from that of the epithelia of the large glands and indeed, biologically, are placed in the group which contains the blood cells.

The relationship between cell division and cell differentiation has been discussed by a number of authors. Bloom (15) states, "Cellular multiplication and cellular differentiation are distinct processes, each of which usually takes place only in the absence of the other." Fischer and Parker (21) say, "Reproductive quiescence is an important factor in differentiation." Weiss (49) says: "Cellular differentiation and cellular multiplication are two processes which, if not strictly mutually exclusive, are nevertheless markedly antagonistic in their tendencies." Dawson (17), however, points out that an apparent inability of cells to divide cannot be accepted as proof of a high degree of specialization. He gives several instances of cells, including those of cartilage, which can be rejuvenated, in the sense of regaining their power to divide even in older animals.

In the present observations on lymphocytes in intestinal epithelium we seem to find a close compatibility between division and differentiation. In fact, a common mechanism of differentiation in the crypts, in our opinion, is by a lymphocyte mother-nucleus dividing to form two epithelial daughter nuclei.

Are there examples of such phenomena which have been firmly established as occurring in any tissues or any animal species? The answer is clearly in the affirmative. The differentiation of the sex cells, through their various stages, is in part a change that occurs with mitosis and, we may well believe, as a result of mitosis. Thus the primary spermatocyte divides to give 2 secondary spermatocytes while they in turn divide to give 4 spermatids.

Among somatic cells, the myeloid elements show mitoses and cell divisions while differentiation is occurring. Actual differentiating mitosis in the somatic cells of vertebrates has been identified only in rare instances. Murray (37) has described in tissue culture of thymic epithelium two instances in which a mitotic division was followed from mother cells in which the original cell was an epithelial cell of thymus while the two daughter cells were an epithelial cell and a thymocyte (small lymphocyte) respectively. This was a culture in which oxygen concentration had been reduced. Differentiation apparently can occur also in a direct way, without mitosis, as Baillif (11) has described abundant formation of thymocytes directly from epithelial cells in the regenerating thymus of the rat. When the findings of these two authors are considered together, we see a condition in the thymic tissue where differentiation from epithelial cell to lymphocyte can occur either directly or through mitosis, much as in the intestine differentiation from lymphocyte to epithelial cells can occur directly

(villi and crypts) or by differentiating mitosis (crypts almost exclusively).

Among the invertebrate animals a number of instances of differentiating mitosis have been described.

It may at first thought seem strange to find so much cell degeneration in the crypts of Lieberkühn and to associate such degeneration with a process of cell differentiation and of cell replacement. If we survey the processes in normal ontogeny, however, we find that cell death is a common occurrence and in fact that, just when actual differentiation of tissues is in process, there are apt to be large numbers of cells which, failing in the attempt to differentiate, will perish. This is the process to which Glücksmann (22) has given the term histogenetic degeneration. Even in the central nervous system such degeneration occurs in many places and at various times during development (20, 29).

Among the lymphocytes in intestinal epithelium the degenerative process seems to be very intimately related to the mitotic division. It would almost seem as though the lymphocyte comes to a fork in the road, where one branch leads to a normal lymphocyte prophase, the other to degeneration by chromatin condensation and eventual dissolution of the nucleus. The many nuclei which seem degenerate and yet at the same time bear some resemblance to nuclei in mitosis lead one to such a view, as does the appearance of many of the nuclei rather definitely in prophase and still showing evidences of possible degeneration.

In the mitotic process the prophase has been shown to be one in which the cell is particularly apt to show the effects of some injurious influences. This has been demonstrated for the effects of radiation on germinative regions of the eye and brain of tadpoles (23, 44, 45); for spermatogonia of mice (38); for the blood-forming cells in tadpoles (42); and for chick fibroblasts in tissue culture (33, 34).

The degeneration of these cells generally occurs in the prophase of the first mitosis following radiation. This probably simply means that the time of reproduction of a cell is to some extent a time of added stress and any injury may manifest itself at such a time. In the case of degeneration of the prophase or near-prophase lymphocytes in the crypts of Lieberkühn, the added effort of differentiating change may be a factor which frequently precipitates degeneration.

It is undoubtedly true that it is not easy to be sure of the identity of a cell once it has reached a certain stage in the mitotic process. The question may be raised as to how we know that the prophases and succeeding stages up to the telophase are generally those of lymphocytes. We have pointed out that the prophase is usually of a ring type, one which could arise readily from a lymphocyte in which chromatin condensation was occurring. Also, we see many probable transition stages between such lymphocytes and these ring prophases, with hardly a case which could be interpreted as a transition from the large vesicular epithelial nucleus to the ring type.

It is interesting to note that Leblond and Stevens (35) counting the mitotic figures in the various stages in the crypts (their table 1, p. 362, on normal rats), came out with as many or more cells in metaphase as in prophase. Since the duration of prophase is known to be far greater than that of metaphase, this seems to point to the probability of a misinterpretation of the stages in their material. Actually, the figure which they present as a metaphase could very well be one of our prophases. In other words, an investigator looking for the more classical picture of an epithelial prophase would be reluctant to accept the numerous ring-shaped figures as prophases and possibly would count as such daughter nuclei in telophase but on separate sections or possibly some interphase epithelial nuclei with variations in internal structure.

What evidence do we have that our own interpretation of the stages of mitosis is correct? And what evidence is there that the majority of mitotic figures are actually those of the lymphocytes?

The evidence for the correctness of our conclusions comes from two main sources 1) the apparent close relationship of the mitotic figures both in their location and their appearance to the intracellular lymphocytes and 2) a comparison of the mitotic figures in the crypts of Lieberkühn with those found in the germinal centers of lymph nodes.

The first source we already have discussed to some extent. It is true, however, that the apical position of the mitotic figures would not be inconsistent with their derivation from epithelial nuclei, since columnar epithelial cells undergoing mitosis in embryonic and other tissues often pull away from their basal connections and round up prior to division. In the crypts, however, the early stages of mitosis show the lightly-staining spheroidal cell body in such close relationship to other epithelial nuclei, deforming them as do the intracellular lymphocytes, that it seems most probable that the nucleus entering mitosis actually is within the cytoplasm of another cell. There usually is but little cytoplasm properly belonging to the intracellular lymphocyte (fig. 9). For the mitotic figures, the cytoplasm is visible and varies in staining capacity from a very light to a color not far different from that of epithelial cytoplasm. This cytoplasm about the nuclear area or the mitotic figure might be derived from the host epithelial cell or might arise through activity of the lymphocyte nucleus—it is not as yet possible to determine its exact origin. It is a matter of great interest to us, however, that in a few cases we have seen lymphocyte nuclei, poised between mitosis and degeneration, with very large, well-stained cytoplasmic bodies. This seems to indicate the ability of the lymphocyte to form a cytoplasmic body comparable to that of an epithelial cell even during this crucial period in its existence (figs. 10 and 11). Of course, we have ample evidence that the non-mitotic lymphocyte can develop such a cytoplasmic body (figs. 5, 6 and 7) during its direct transformation to an epithelial cell.

In earlier papers (1, 5) we have described the occurrence in the germinal (reaction) centers of the spleen and of lymph nodes of mitotic division and of degeneration of lymphocytes as phenomena constantly occurring together in normal animals. Recently, we have reviewed some of our material. It is clear that in these sites the course of mitosis is from interphase lymphocyte nuclei through nuclei with condensed chromatin and on to a ring-shaped prophase, just as in the case of the mitoses in the crypts of Lieberkühn.

The concept of lymphoepithelial metamorphosis is one which of necessity should lead to new ways of thinking about many aspects of cell relationships. Still,

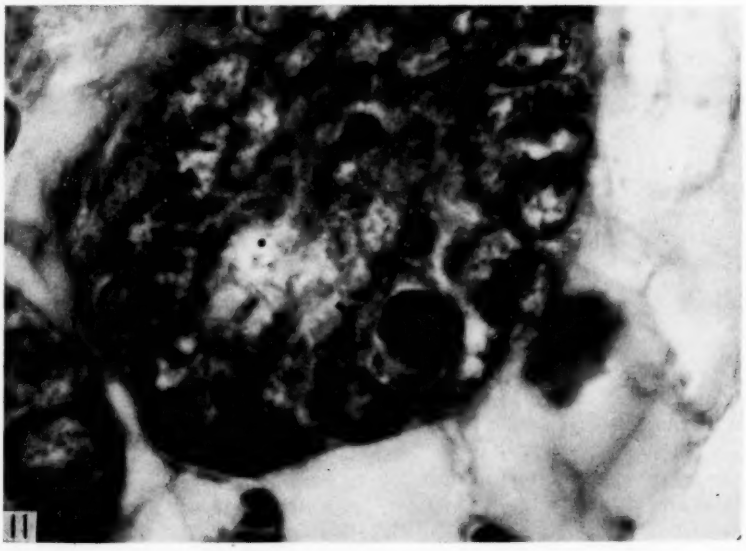


Fig. 10. An intracellular lymphocyte in the epithelium of a crypt of a 784-day male mouse. This figure illustrates the close similarity in appearance of lymphocytes undergoing degeneration by chromatin condensation and of mitotic figures in the crypts. Here the chromatin arrangement is strongly suggestive of a metaphase but the nuclear membrane (arrow) seems to be intact. The nucleus of the epithelial cell in which the lymphocyte lies is below and to the left of the lymphocyte which itself is remarkably large. X1995.

Fig. 11. The same field as in figure 10 at a slightly different focus. Here the outline of the lymphocyte body can be seen clearly (arrow). The cytoplasm of this dividing cell appears very similar to that of the epithelial cells but its eosinophilic nucleus containing condensed chromatin and its relationship to the epithelial cells make its originally lymphocytic nature, in our opinion, a matter of no doubt. X 1995.

it actually is in accord with much of our knowledge of the lymphocyte (50).

Studies both on fixed material and on tissue cultures have indicated that lymphocytes may transform into macrophages (18, 31, 36); fibroblasts (14), polymorphonuclear leucocytes (16, 30), and plasma-cells (5).

While it would be wrong to say that such conclusions have gone undisputed, still the evidence has come from many types of study. The multipotential character of the lymphocyte seems to have been firmly established.

The ability of the lymphocytes to transform into epithelial cells of surface layers, however, must focus our attention still more sharply on this multipotential cell type and its relationship to the great problems of the aging process in the community of cells which constitutes the organism.

SUMMARY

1. The small intestine (jejunum) of young adult and of senile mice of the C57 Black strain has been studied in regard to (a) the general tissue constituents of the intestinal wall and (b) the details of cellular relationship in the epithelium of the crypts of Lieberkühn and of the villi, with special attention to the role of the lymphocyte.

2. In old age the development of a coarse-fibered connective tissue with a paucity of cells is a conspicuous feature in the tunica propria.

3. The crypts frequently are widely separated by the newly-formed connective tissue. In the cores of the villi, on the other hand, it seems to form either not at all or very rarely.

4. An instance is described in which a further differentiation occurs in the connective tissue, the appearance of basophilic spicule-like structures which contain cells in lacunae. This occurred in the oldest animal of the series.

5. In this series of animals, migrating lymphocytes in the epithelium are, in general, more abundant in the young than in the old animals.

6. There is evidence at all ages of a direct transformation of lymphocytes into epithelial cells on the villi and in the crypts.

7. The intracellular position of many lymphocytes in the crypts is a constant finding in young and old animals. A peculiar process of degeneration by chromatin condensation is described. Degenerating lymphocyte nuclei often resemble mitotic figures, particularly prophases.

8. The abundant mitotic figures in the crypts, generally heretofore described as epithelial cells in mitosis, are interpreted by us as, at least for the most part, lymphocytes which are undergoing a differentiating mitosis to give rise to new epithelial cells.

9. The concept of the multipotential nature of the lymphocyte and of its importance in the community of cells is greatly widened by the conclusions from this study.

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A COMPARISON OF INTRACELLULAR NITROGEN AND METAL ION DISTRIBUTION PATTERNS IN THE LIVERS OF YOUNG AND OLD RATS*

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Studies from this laboratory have recently been reported (7) showing that potassium, sodium, calcium, and magnesium are distributed among the subcellular components of the rat liver cell in a manner which cannot be attributed to free diffusion. Furthermore, these distribution patterns differ for each of the metal ion species studied. In view of the possible importance of metal ions, especially calcium, in the aging process (9), it was decided to compare these distribution patterns in young and old rats. As a part of this investigation, the partition of total nitrogen between cell components was also measured, and the results are reported herein.

METHODS

Rat liver cell fractions were prepared by the method of differential centrifugation (16, 17). The homogenized liver, suspended in 0.25 M sucrose solution, was centrifuged for 15 min. at 600 × gravity. The washed sediment was designated the *nuclear fraction*. The *mitochondrial fraction* was prepared by centrifuging the supernatant fluid for 7-8 minutes at 17,300 × gravity. The mitochondrial sediment was washed twice. The third particulate component, the *microsomal fraction*, was obtained by centrifuging the remaining material at 102,750 × gravity for 30 min. and washing the sediment once. The fluid remaining from the above was designated the *supernatant fraction*. At each step, all washings were added to the corresponding supernatant fluid so that no material was lost. The various fractions were not pure preparations, but it was necessary to accept impurity in order to arrive at a complete balance sheet for the cell as a whole. The fractionation method is described in more detail elsewhere (7).

Nitrogen determinations were performed by Kjeldahl digestion and titration (12).

Potassium and sodium were measured on solutions of ashed samples by internal standard flame photometry.

Calcium and magnesium were separated on an ion exchange column (8) and titrated by the versenate method of Biedermann and Schwarzenbach (2) on a microgram scale.

The animals in this study were two groups of Long-Evans male rats, young adults, ranging in weight from 184 to 270 Gm., and old, from 456 to 610 Gm. These latter rats were known to be at least 2 years old.

RESULTS AND DISCUSSION

Nitrogen: Table 1 shows the results of nitrogen analyses of the cell fractions, expressed as the percentage of the whole homogenate nitrogen found in each cell fraction. Each figure is the mean of the analyses of from 5 to 7 livers. The probabilities of the differences found between young and old groups being due to chance were calculated by application of the "t" test (4), and these probabilities are given in the table in the column headed "P."

It is seen that nitrogen is distributed differently among the cell fractions of the young and old animals. The old rats have a significantly higher proportion of nitrogen in the supernatant fraction and a lower proportion in the mitochondrial fraction than is found in the young. Since the mitochondria are the seat of the oxidative metabolism of the cell, one would expect that a reduction in the quantity of mitochondrial material would be reflected in a diminished respiratory activity. Such a change with age has been observed by some workers, but not by others. For instance, Belasco (1) found a decrease in oxygen uptake by male rat liver slices of 26 per cent between 4 and 12 months of age. In another series by the same worker the decrease was 10 per cent. Pearce (13) reported decreases with age of the same order of magnitude in the liver of the mouse.

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TABLE 1. NITROGEN PARTITION IN LIVER CELL FRACTIONS OF YOUNG AND OLD RATS. FIGURES ARE PERCENTAGES OF HOMOGENATE NITROGEN FOUND IN CELL FRACTIONS.

Cell Fraction	Young (%)	Old (%)	P
Nuclear	15.0	16.5	>.05
Mitochondrial	26.2	20.7	<.01
Microsomal	36.9	32.3	>.05
Supernatant	17.7	25.8	<.05
Recovery	95.8	95.3	

On the other hand, Reiner's data (14) on rats show a decrease with age in the oxygen uptake of liver homogenate, but the author did not consider the difference to be a significant one. In a study by Ross and Ely (15), a decrease with age in the oxygen uptake of rat liver slices was noted, but this was changed to a slight increase by the addition of succinate to the system. The other studies cited above used glucose as substrate. Ross and Ely (15) also made mitochondrial counts by microscopic examination and found no change with age. The mitochondrial volumes and nitrogen contents, however, were not measured.

A difference similar to ours in nitrogen partition was found between mouse liver and hepatoma by DuBuy, Woods, and Showacre (6). In their preparations, a ratio (calculated from their table 1) of 0.67 was found between the nitrogen concentrations of the particulate material and the supernatant fraction of normal liver, while fractions prepared under similar conditions from a mouse hepatoma gave a ratio of 0.42. The parallel between the case of the tumor with its impaired oxidative metabolism and the senescent liver is an evident one.

TABLE 2. METAL ION PARTITION IN LIVER CELL FRACTIONS OF YOUNG AND OLD RATS. FIGURES ARE MICROEQUIVALENTS OF METAL ION PER MILLIGRAM OF NITROGEN.

Cell Fraction	Potassium		Sodium		Calcium		Magnesium	
	Young	Old	Young	Old	Young	Old	Young	Old
Homogenate	1.21	1.24	0.400	0.372	0.029	0.023	0.166	0.152
Nuclear	0.87	0.93	0.308	0.264	0.032	0.022	0.167	0.148
Mitochondrial	0.87	0.89	0.124	0.089	0.055	0.051	0.147	0.144
Microsomal	0.35	0.35	0.147	0.144	0.022	0.022	0.221	0.178
Supernatant	3.67	3.05*	1.45	1.10	0.065	0.025*	0.122	0.099*

* P <.05

The present finding of a decrease of mitochondrial nitrogen with age, accompanied by a comparable increase in the nitrogen of the supernatant fraction, might be interpreted as an artifact of the frationation procedure, postulating a greater fragility of the mitochondria of aged animals, resulting in their disruption due to mechanical or osmotic effects, and the appearance of their proteins in the soluble fraction. However, much of the mitochondrial protein is notably difficult to solubilize, and it is hard to believe that such mitochondrial fragments would appear in the supernatant without also appearing in the microsomes (3).

Metal Ions: The mean values of the concentrations found for the 4 metal ions in the homogenate and the cell fractions are presented in table 2. Adding the quantities of each metal ion found in the cell fractions and comparing these to the amount found in the homogenate gave the following average percentage recoveries: potassium, 98; sodium, 100; calcium, 106; magnesium, 97.

P values for the differences between young and old groups were calculated, and a scattering of significantly small values (at the .05 level) was found, as indicated by asterisks. All these are in the supernatant fraction. However, because they are unaccompanied by significant differences in concentrations in the whole homogenate, it is questionable whether any real importance can be attributed to them. It may be noted in this connection that it is a property of statistical tests that in any such series of calculations, a few "significant" differences will be found, in the absence of any "true" differences (5).

An increase in calcium has frequently been mentioned as a concomitant of the aging process, particularly by Lansing (9, 10), who considers it to be a phenomenon of widespread occurrence throughout the plant and animal kingdoms. Concerning the hepatic metal ions specifically, there seems to be a difference between species. Simms and Stolman (18) found increases in the hepatic concentrations of sodium, potassium, calcium and magnesium in aged humans, while Lowry and associates (11), working with rats, found no changes. The present study is consistent with the latter, and extends this finding of lack of differences to the subcellular level.

SUMMARY

Cell fractions of young and old rat liver were prepared by differential centrifugation and analyzed for their content of nitrogen, potassium, sodium, calcium, and magnesium.

Old rats showed a lower proportion of liver-cell nitrogen in the mitochondria and a higher proportion in the supernatant fraction compared to young adult rats. This observation may possibly be related to the lower respiratory rate which others found in the livers of old rats.

No significant differences have been found in the metal ion distribution patterns between young and old rats.

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THE PROTEIN COMPOSITION OF SPONGE-BIOPSY CONNECTIVE TISSUE WITH SPECIAL REGARD TO BIOPSY-TISSUE AGE

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Connective tissue, which supports all arteries and veins, constitutes an important tissue for the investigation of the aging and the atherosclerotic processes. For this reason, research in our laboratories has been directed toward the study of the biochemical and the physical chemical properties of connective tissue. This report on the protein composition of connective tissue and on the nature of this protein is a phase of these studies.

Connective tissue procured by the sponge-biopsy technique previously described (2) was used throughout the study. This technique involves the implantation subcutaneously of a small square of polyvinyl sponge (Ivalon)† with subsequent removal. Serial histologic examination of the connective tissue obtained indicated an early minimal degree of reaction such as giant cells or round cell infiltration which disappeared with time. Normal appearing connective tissue was found filling the pores of the implanted sponges. Early work indicated that the saline-soluble globular protein can be separated from the saline-insoluble fibrous protein. The saline-soluble globular proteins have an amino acid composition similar to that reported for ground substance, while the saline-insoluble protein contains all the tissue hydroxyproline, the amino acid hallmark of collagen (3). The lipids of biopsy-connective tissue from various species have been quantitated and reported from our laboratory (2, 3, 4, 17).

In the present paper a report will be given of chemical analyses and electrophoretic studies on various fractions of connective tissue-sponge biopsies obtained from rats and human subjects. Special consideration will be given to the effect of biopsy-tissue age upon its protein composition.

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† Ivalon was supplied by Clay-Adams, Inc.

METHODS

The connective tissue was weighed and homogenized in physiologic saline as previously reported (3). The saline-insoluble fraction was then exposed to testicular hyaluronidase (300 viscosity units)‡ under toluene for 3 days at 37 C. in an acetate buffer, pH 5.2, containing 0.15 M of sodium chloride (13) (table 1 procedure A). The insoluble residue from the hyaluronidase treatment was allowed to stand at room temperature with 0.1 N sodium hydroxide overnight and the alkali-soluble fraction removed by centrifugation.

In order to obtain a solubilized collagen, the non-collagenous proteins were removed by homogenizing in 0.5 M sodium acetate at 6 C. (9), and the resultant scleroprotein was then extracted in 0.5 M acetic acid at 6 C. for 24 hours (10) (table 1 procedure B).

A list of the different fractions obtained by the procedure employed is given in table 1.

Protein, hexosamine, and lipid determinations were carried out on the saline-soluble (I), hyaluronidase-liberated (IIa), alkaline-soluble (IIb), and residual fraction (IIc). The analyses were performed on 6 pooled 36-day-old biopsy specimens and on 3 pooled 120-day-old specimens from the rat. In addition, measurements were made on 12 pooled human biopsy-tissue specimens, which had an age of approximately 120 days. The nitrogen determinations were carried out by the microdiffusion technique of Conway (6) following Kjeldahl digestion (11). Hexosamine was quantitated by a modification of the method of Elson and Morgan (8) and the total lipid was analyzed by a modified serum turbidimetric method (7).

Amino acid analyses were performed on hydrolysates of the saline-soluble and total saline-insoluble fractions. Connective tissue biopsies of 60 to 160 days' growth from 9 adult rats and of 110 to 140 days' growth from 8 adult hu-

‡ Alidase (brand of hyaluronidase) was furnished for these studies by G. D. Searle and Company.

TABLE 1. FRACTIONATION PROCEDURES.

Procedure A
I. Saline-soluble fraction
II. Saline-insoluble fraction
  a. Hyaluronidase-liberated fraction
  b. Hyaluronidase-resistant fraction solubilized with NaOH
  c. Residual scleroprotein

Procedure B
I. Sodium acetate-soluble fraction
II. Sodium acetate-insoluble fraction
  a. Acetic acid-soluble collagen
  b. Residual scleroprotein

Procedure C
I. Sodium citrate-citric acid soluble fraction
II. Sodium citrate-citric acid insoluble fraction
  a. Alkali-soluble
  b. Residual fraction

mans of both sexes were used in the amino acid analyses.

Eighteen amino acids were determined quantitatively by microbiologic assay (12). *Streptococcus faecalis R* (ATCC 8043) was used for methionine, arginine, histidine, and threonine; *Leuconostic mesenteroides* strain p-60 (ATCC 8042) for isoleucine, proline, tyrosine, lysine, aspartic acid, glycine, serine, and cystine, and *Lactobacillus plantarum* (arabinosus) strain 17-5 (ATCC 8014) for leucine, phenylalanine, valine, glutamic acid, alanine, and tryptophan. Glutamine was added to each tube for the glutamic acid assay (14). Vitamin B_6 was omitted from the medium in the assay of alanine (15). Hydroxyproline was determined colorimetrically (16).

Electrophoretic studies were carried out on the saline-soluble (AI), hyaluronidase-liberated (AIIa), alkali-soluble (AIIb), sodium acetate-soluble (BI), acetic acid-solubilized (BIIa) fractions of the rat biopsy-tissue, and on the saline-soluble fraction (AI) of human biopsy-tissue. Connective tissue biopsies of 36 to 120 days' growth from 8 adult albino rats and of 120 days' growth from 9 humans of both sexes ranging in age from 25 to 40 years were used for this study. For the electrophoretic studies it was found necessary to desalt the saline-soluble, hyaluronidase-liberated and alkali-soluble fractions by passage through an Amberlite monobed column.* The desalted fractions were evaporated almost to dryness in a vacuum desiccator containing phosphorus pentoxide and then applied to paper strips which had been wet with a barbiturate buffer, pH 8.6 and ionic strength 0.075, and drained. Twenty to 30 microliters of sample, which contained 1-4 mg. protein, were applied.

For electrophoresis of the acetic acid-solubilized collagen (BIIa) the acetic acid supernatant was clarified by ultracentrifugation at 29,000 rpm for 2½ hours, and 50 microliters, containing 50-100 micrograms of protein, were applied to a paper electrophoretic strip saturated with an acetate buffer, pH 3.5 and ionic strength 0.05.

The Spinco paper electrophoresis cell (Durrum type) was run either with a current of 10 ma. for 8 hours or 5 ma. for 16 hours at 6 C. for acid-soluble collagen and at room temperature for serum and all other tissue fractions. The proteins, except collagen, were stained with bromphenol blue solution (21), the carbohydrate with a periodic acid and fuchsin bisulfite reagent (1), and the lipid with Oil Red 0 (1). The collagen strips were dyed in a solution containing 0.25 Gm. aniline blue and 2 cc. glacial acetic acid per 100 cc. distilled water, rinsed with running distilled water, then 1 per cent acetic acid for 3 min., 95 per cent ethanol for 1 min., and dried in air. With each electrophoretic analysis, a parallel sample of serum served as a standard reference. The concentration of each component on the elec-

TABLE 2. PROTEIN, HEXOSAMINE, AND LIPID CONCENTRATIONS OF BIOPSY-CONNECTIVE TISSUE (RAT AND HUMAN) (Gm. per 100 Gm. wet tissue).

	Number of Tissue Samples	
	Rat, 9	Human, 12
Saline-Soluble Fraction (AI)		
Protein	4.40	6.05
Hexosamine	0.15	0.27
Lipid	0.94	2.34
Hyaluronidase-Liberated Extract (AIIa)		
Protein	0.90	—*
Hexosamine	0.03	0.11
Lipid	—	—
Alkali Extract (AIIb)		
Protein	0.50	1.54
Hexosamine	0.04	—
Lipid	—	—
Residue (AIIc)		
Protein	3.20	8.91
Hexosamine	0.03	0.11
Lipid	1.17	1.99
Total		
Protein	9.00	16.50
Hexosamine	0.25	0.49
Lipid	2.11	4.33

* Not determined.

* Amberlite MB-1 was provided through the courtesy of Rohm and Haas Company.

TABLE 3. AMINO ACID COMPOSITION OF BIOPSY-CONNECTIVE TISSUE (RAT AND HUMAN) (GM. PER 100 GM. PROTEIN).

	Saline-Soluble Fraction		Saline-Insoluble Fraction	
	Number of Tissue Samples		Number of Tissue Samples	
	Rat, 9	Human, 8	Rat, 9	Human, 8
Amino Acid				
Neutral:				
Glycine	2.55 ± 0.51	1.52 ± 0.53*	18.76 ± 3.25	13.55 ± 3.00*
Alanine	10.32 ± 0.95	10.19 ± 3.13	8.94 ± 1.54	8.42 ± 2.53
Serine	11.46 ± 2.61	8.96 ± 1.81	11.83 ± 5.05	9.81 ± 1.52
Valine	16.87 ± 2.43	13.82 ± 2.19*	8.86 ± 1.33	10.54 ± 1.52*
Threonine	11.67 ± 1.75	7.29 ± 1.30*	6.56 ± 0.93	6.46 ± 0.45
Isoleucine	11.75 ± 2.40	10.16 ± 2.01	6.46 ± 1.13	8.75 ± 0.82*
Leucine	11.35 ± 1.46	11.25 ± 2.37	6.46 ± 1.09	8.42 ± 1.37*
Acidic:				
Aspartic acid	11.17 ± 2.96	11.87 ± 1.88	8.89 ± 1.13	9.42 ± 1.22
Glutamic acid	15.01 ± 5.36	18.20 ± 5.50	10.13 ± 2.08	19.34 ± 4.87*
Basic:				
Arginine	6.87 ± 1.20	4.53 ± 0.56*	10.10 ± 2.18	8.07 ± 0.62*
Lysine	13.66 ± 2.29	17.47 ± 2.65*	10.51 ± 1.76	11.81 ± 1.56
Histidine	3.45 ± 0.55	2.72 ± 0.79*	0.43 ± 0.14	1.03 ± 0.28*
Sulfur-Containing:				
Cystine	0.66 ± 0.44	1.62 ± 0.81*	0.22 ± 0.12	0.79 ± 0.44*
Methionine	2.85 ± 1.32	2.84 ± 0.85	1.48 ± 0.69	2.70 ± 0.48*
Aromatic:				
Proline	3.26 ± 1.31	2.22 ± 0.71	11.00 ± 0.91	8.14 ± 1.87*
Hydroxyproline	0.00	0.00	7.92 ± 2.05	6.51 ± 0.94
Tyrosine	3.36 ± 1.22	1.71 ± 0.91*	0.00	0.00
Tryptophan	0.18 ± 0.03	0.36 ± 0.20	0.04 ± 0.07	0.97 ± 0.04*
Phenylalanine	11.61 ± 1.85	9.18 ± 0.77*	5.22 ± 0.48	6.68 ± 0.61*

Mean values and standard deviations.
* $p < 0.05$ in comparison between rat and human values of a fraction.

trophoretic strip was determined with the aid of the Spinco Analytrol scanning device.

A special study was made on the effect of biopsy-tissue age on the distribution of non-collagenous and collagenous protein (table 1, procedure C). For this investigation the fresh sponge-biopsy was sliced and shaken with a citrate buffer, pH 4.3, containing 0.1 M each of sodium citrate and citric acid for 24 hours at 6 C. (9). After centrifugation in the cold and removal of the citrate buffer supernatant, the residue was shaken with 0.1 N sodium hydroxide for 24 hours at 6 C. The total protein and hydroxyproline concentrations of the citrate-soluble, alkali-soluble, and residual fractions were determined. Eight 10-day tissues and 5 180-day tissues of adult male and female rats were analyzed.

RESULTS

The protein, hexosamine, and lipid concentrations of the saline-soluble, hyaluronidase-liberated, alkali-soluble (room temperature), and tissue residue fractions are shown in table 2. It is immediately apparent that the protein of the tissue was divided principally between the saline-soluble and the residue fractions. Some protein was solubilized by the hyaluronidase treatment and a smaller amount by the alkali treatment. The protein concentration in the human connective tissue (16.5 Gm. per cent) was far greater than in the rat tissue, and the bulk (54 per cent) was located in the residue fraction.

In biopsy-connective tissue of the rat and man, hexosamine was found chiefly in the saline-soluble fraction. The remainder of the hexosamine was about equally divided among the other fractions. Larger amounts of hexosamine were found in the hyaluronidase-liberated and residue fractions of human tissue than in these fractions of rat tissue.

Lipids were determined only on the saline-soluble and the -insoluble fractions and were equally distributed between the two.

Amino Acid Composition of Saline-Soluble and Saline-Insoluble Fractions.—The amino acid composition of the sponge-connective tissue of the rat and human is shown in table 3. The saline-insoluble fraction of the connective tissue contained all of the tissue hydroxyproline and larger concentrations of glycine and proline, amino acids which characterize collagen. The low concentrations of glycine and proline and the high concentration of tyrosine indicated that the saline-soluble fraction contained the non-collagenous protein of the ground substance and protoplasma. Tyrosine was not found in the saline-insoluble fraction of the sponge-connective tissue of either the human or rat. Both fractions of the connective tissue of the rat had higher concentrations of glycine and arginine and a lower concentration of cystine than the tissue of the human. The saline-insoluble fraction of the connective tissue of the rat had significantly higher concentrations of proline, glycine, and arginine and significantly lower concentrations of valine, isoleucine, leucine, glutamic acid, histidine, cystine, methionine, trytophan, and phenaylalanine than a similar fraction of human connective tissue. The saline-soluble fraction of rat connective tissue had higher concentrations of glycine, valine, threonine, arginine, histidine, tyrosine, and phenylalanine and lower concentrations of lysine and cystine than the saline-soluble human tissue fraction.

In a comparison of the amino acid composition of 60-day-old biopsy-connective tissue with 160-day-old tissue of the rat, several differences in the saline-soluble and total saline-insoluble fractions were found. The older male biopsy-connective tissue had higher concentrations of methionine and cystine in the saline-insoluble fraction and a higher concentration of lysine and lower concentrations of proline and phenyalanine in the saline-soluble fraction. The only difference observed in the female tissue was a larger concentration of cystine in the saline-insoluble fraction of the older tissue.

Electrophoretic Study of Tissue Fractions.—Migration of the protein of the various fractions of rat biopsy-connective tissue in an electric field is depicted in figure 1. An electrophoretic pattern for rat serum is shown at the bottom of the figure to identify the protein peaks. Samples were applied at the points indicated by the short line below each pattern. In general, the saline-soluble fraction resembled the serum in electrophoretic protein pattern, i.e., albumin, alpha, beta, and gamma globulin. The trace amounts of protein in the hyaluronidase-liberated fraction were only faintly stained in the region of albumin. A large single protein band with a mobility similar to alpha$_1$ globulin was observed in the electrophoretic strip of the alkali extract. The electrophoretic patterns of the saline-soluble and alkali-soluble fractions of human connective tissue were similar to those shown for rat tissue. The mobilities of the serum and saline-soluble proteins of connective tissue were found to be similar for a species. These protein components of human serum and connective tissue had greater mobilities than those of the rat.

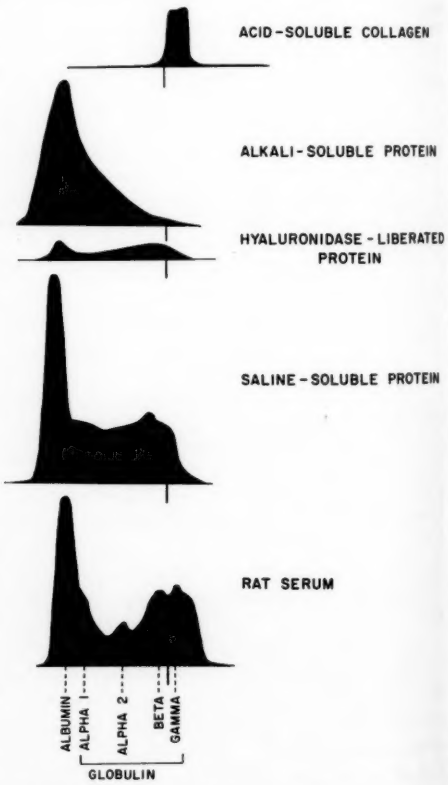

Fig. 1. Electrophoretic patterns of biopsy-connective tissue and serum of the rat.

TABLE 4. ELECTROPHORETIC ANALYSES OF SERUM AND BIOPSY-CONNECTIVE TISSUE (RAT AND HUMAN) PERCENTAGE.

Serum and Number of Samples Analyzed	Albumin	Globulin			
		Alpha$_1$	Alpha$_2$	Beta	Gamma
Protein—Rat (5)..... (A)	28.3* ±6.9	14.7 ±2.7	16.1 ±8.4	19.8 ±2.3	21.1 ±3.3
Protein—Human (6)..... (B)	54.3 ±5.3	5.8 ±1.6	10.2 ±2.0	13.7 ±3.7	16.4 ±1.6
A : B	†	†		†	†
Lipid—Rat (5).....	20.8 ±10.3	47.5 ±19.4		31.5 ±8.7	0.0
Carbohydrate—Rat (5)...	18.5 ±3.8	46.2 ±9.5		35.5 ±5.6	0.0
Connective Tissue and Number of Samples Analyzed					
Saline-Soluble Fraction					
Protein—Rat (9)..... (C)	44.0 ±10.8	27.2 ±14.2	0.0	21.5 ±3.4	7.2 ±2.2
Protein—Human (8)..... (D)	36.9 ±13.1	12.8 ±3.9	18.0 ±6.9	18.5 ±6.1	14.0 ±4.2
C : D		†			†
A : C	†				†
B : D	†	†	†		
Carbohydrate—Rat (9)...	18.1 ±6.5	40.8 ±11.7	0.0	40.9 ±3.2	0.0

* Mean value and standard deviation.
† $p < 0.05$.

The electrophoretic distribution of the conjugated proteins of rat serum and of connective tissue extracts is recorded in table 4. Electrophoretic strips of rat serum, which were stained for protein, lipid, and carbohydate, revealed that a union exists among the protein, lipid, and carbohydrate at the site of the albumin and beta globulin. The mobile lipid and carbohydrate in the area of alpha$_1$ and alpha$_2$ globulins were not clearly delineated to either of these zones. However, the lipid and the carbohydrate of the serum were chiefly bound to the globulins. Four protein electrophoretic zones, albumin, alpha$_1$, beta, and gamma globulins, were found in the saline-soluble fraction of rat connective tissue. The protein band at the alpha$_2$ globulin area was consistently absent in the saline-soluble fraction. The carbohydrate was localized principally in the zones of alpha$_1$ and beta globulins. Even though a considerable amount of lipid was found in the saline-soluble fraction (3), none of it migrated from the point of application under these experimental conditions. The hyaluronidase-liberated fraction of rat connective tissue contained only a trace of protein, which migrated to the serum albumin position. The large protein zone of the alkali-soluble fraction moved to the alpha$_1$ globulin position and carried with it a prominent carbohydrate and a small lipid moiety.

Acetic acid-solubilized collagen moved a short distance from the origin toward the cathode in an acetate buffer of pH 3.5. A small amount of carbohydrate and lipid migrated with the solubilized collagen. In buffers of higher pH, i.e., 4.8 and 8.6, the solubilized collagen remained at the point of application.

In general, the protein and carbohydrate components of all the soluble fractions of human biopsy-connective tissue were qualitatively similar to those of rat biopsy-connective tissue. The saline-soluble fraction of human connective tissue had electrophoretic zones corresponding to the serum bands for albumin, alpha$_1$, alpha$_2$, beta, and gamma globulins. The outstanding species difference between the saline-soluble tissue fractions of the two species was the presence in the human tissue of a protein in the region of alpha$_2$ globulin. No species differences were noted in the protein, carbohydrate or lipid electrophoretic distribution in the alkali extract or the acetic acid-solubilized collagen.

In table 4, the electrophoretic distribution of the proteins in the saline-soluble connective tissue fractions and in sera of rat and human are compared. Human serum had a greater concentration of albumin and smaller concentrations of alpha$_1$, beta, and gamma globulins than rat serum. There was less alpha$_1$ globulin and more gamma globulin in the saline-soluble fraction of human connective tissue than in that of the rat. The presence of alpha$_2$ globulin in the saline-soluble extract of human connective tissue and its absence in the rat has been alluded to in the above paragraph.

The age of the connective tissue biopsy affected the sharpness of the electrophoretic separation of the saline-soluble proteins. Adult rat tissue of 36 days of age had prominent albumin, alpha$_1$, beta, and gamma globulin

TABLE 5. THE EFFECT OF TISSUE AGE ON THE DISTRIBUTION OF PROTEIN IN BIOPSY-CONNECTIVE TISSUE (RAT).

Tissue Fraction	Percentage of Total Protein			Hydroxyproline/Protein × 100		
	10-day C. T.-sponge (8)*	180-day C. T.-sponge (5)*	p‡	10-day C. T.-sponge (8)*	180-day C. T.-sponge (5)*	p‡
Citrate extract (CI)	57.68 ± 2.90†	25.46 ± 3.74	< 0.001	0.68 ± 0.24	1.06 ± 0.36	< 0.05
Alkali extract (CIIa)	17.97 ± 2.60	25.58 ± 4.03	< 0.05	0.21 ± 0.04	0.46 ± 0.24	< 0.02
Residue (CIIb)	24.27 ± 1.84	46.98 ± 2.32	< 0.001	6.72 ± 0.54	7.89 ± 0.70	< 0.02

C.T. = Connective tissue.
* Number of animals.
† Mean value and standard deviation.
‡ p = Probability of a chance occurrence of the difference.

electrophoretic bands. Strips for this fraction of the 120-day-old tissue, however, had blurred bands with a heavily stained background. The amount of protein which failed to migrate from the point of application was greater in older biopsy-tissue. Age of the biopsy-connective tissue did not affect the electrophoretic distribution in the various soluble tissue fractions.

The results of this work did not disclose any sex dependent electrophoretic characteristic of the several tissue fractions studied.

Effect of Biopsy-Tissue Age on the Distribution of Protein in Connective Tissue Fractions.—In table 5, the distribution of total protein and hydroxyproline among the citrate extract, the alkali extract, and the residue is compared for rat connective tissue biopsies of 10 and 180 days' growth. It is apparent that the proteins of the citrate and alkali extracts, which contained only small amounts of hydroxyproline, were primarily non-collagenous and that the residue was composed principally of collagenous protein. The hydroxyproline concentration of each fraction of the 180-day-old sponge-connective tissue was significantly higher than in the corresponding fraction of the 10-day-old sponge connective tissue. In the 10-day-old tissue, the collagenous protein concentration was about 25 per cent of the total protein, while in the 180-day-old tissue it was approximately 50 per cent.

DISCUSSION

Biopsy-connective tissue has been fractionated into saline-soluble, hyaluronidase-liberated, alkali-soluble, and residue fractions, which are chemically different. The protein and lipid of adult rat and human connective tissue are chiefly distributed between the saline-soluble and residue fractions; hexosamine is found principally in the saline extract.

From the results of the electrophoretic study it seems that there is a general similarity between the mobilities of the proteins of the serum and saline-soluble fraction of biopsy-connective tissue within a species. This was observed in the rat, man, dog, rabbit, and guinea pig. However, there are marked differences in the percentage distribution of a protein component between the serum and saline-soluble tissue fraction (table 4). In the rat, there is a greater amount of albumin and a smaller amount of gamma globulin in the saline-soluble protein of the connective tissue than in the serum. The saline-soluble protein of human connective tissue contains a larger amount of $alpha_1$ and $alpha_2$ globulins and a smaller concentration of albumin than the serum. These differences in protein distribution between the saline-soluble fraction of connective tissue and the serum suggest that the proteins of the saline-soluble fraction are not the result of a simple filtration of the serum proteins.

It is of interest to note that biopsy-tissue age affects the distribution of protein between the soluble and insoluble fractions of rat connective tissue. As noted in table 5, there is a higher percentage of non-collagenous protein (citrate and alkali extracts) in the young 10-day-old biopsy than in the 180-day-old tissue. This finding is in accord with the histologic observation that there is more ground substance in younger than in older biopsy-connective tissue.

In the saline-soluble fraction of connective tissue there is a large amount of lipid but it

does not migrate with any of the protein components. The failure of lipid to migrate with protein seems to suggest that the lipid in the saline-soluble fraction was only in suspension or was weakly bound to the protein.

It has been observed that as sponge-connective tissue ages, the concentration of lipids increases significantly (17). Connective tissue of man and chicken has been found to be rich in lipids (4), and electrophoretic patterns for the saline-soluble fraction of the connective tissue of these species and for the older tissues are difficult to interpret because of excessive background staining and lack of distinct protein bands. This may indicate an effect of lipid upon the migration of the tissue protein which may be more than a passive phenomenon. The reduction in the sharpness of the electrophoretic zones may be related to the presence of large amounts of neutral fat since the bulk of chicken and human connective tissue lipids is neutral fat and since the lipids of rat connective tissue which increase with tissue age are neutral fat and cholesterol (17).

Electrophoretic studies reveal that the alkali extract contains a single protein which migrates to the alpha, globulin position and carries with it a large carbohydrate and a small lipid component. This protein has a very low hydroxyproline concentration and may represent, as suggested by others (18), an intermediate between the globular protein of the saline-soluble fraction and the fibrous scleroprotein of the saline-insoluble fraction.

The acetic acid-solubilized collagen of the rat and human biopsy-connective tissue moves slowly toward the cathode and carries small amounts of carbohydrate and lipid. This is of interest since collagen has been thought to be primarily a helical polypeptide chain (19) made up of regularly recurring amino acids (20). It is apparent from these studies that the acetic acid-solubilized collagen contains in addition to the protein a carbohydrate and lipid moiety.

Amino acid analyses indicate that saline homogenization separates non-collagenous protein from collagen. The amino acid composition of collagen of the sponge-connective tissue seems to differ from that reported for other collagenous tissues (5) in the absence of tyrosine, the presence of trace amounts of tryptophan and cystine, and a lower hydroxyproline concentration.

The saline-soluble protein of connective tissue and serum protein are found to have similar amino acid composition (3), i.e., an absence of hydroxyproline, low concentrations of glycine, the sulfur-containing amino acids, tryptophan and histidine, and high concentrations of glutamic acid, lysine and leucine. In spite of their similar amino acid composition, the proteins are quantitatively different.

Significant differences which are observed between the amino acid compositions of the residue and saline-soluble fractions of rat and of human biopsy-connective tissue may be related to the quantitative protein differences of the two tissue extracts.

SUMMARY

Connective tissue-sponge biopsies of adult rats and humans were fractionated into several soluble and insoluble components. These fractions were analyzed chemically and electrophoretically.

It was found that the age of the connective tissue biopsy affects the distribution of protein between the soluble and insoluble fractions in that the percentage of soluble protein decreases in older tissues.

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THE INFLUENCE OF AGE AND OF RENAL ISCHEMIA AT DIFFERENT AGE LEVELS ON RENAL ALKALINE PHOSPHATASE IN THE RAT*

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Alkaline phosphatase is found in many tissues, but even where it exists in high concentration such as in liver and kidney its physiologic significance is unknown. Kay (6) reported that renal alkaline phosphatase concentration in rabbits rises during the first 2 to 3 weeks of life and declines in old age. Brain reported (2) that renal alkaline phosphatase concentration in human infants and fetuses is considerably lower than in older individuals and that it falls in chronic nephritis in man and in uranium nitrate induced nephritis in rabbits. Others have confirmed (3, 15, 16) and denied (5) that a fall in renal alkaline phosphatase activity occurs in experimental nephritis. Phosphatase is said to disappear from kidneys autolyzing after ligation of the renal pedicle (7) or developing hydronephrosis (4, 17). The relationships between renal alkaline phosphatase and various endocrine states have also been studied (8, 9, 13, 14).

In the present study we have determined in rats the renal alkaline phosphatase activities present in normal kidneys and at stated intervals after complete occlusion of the renal artery. The mean activities for a younger and an older group have been compared.

METHODS

Normal rats (184) of the Purina and Sprague Dawley strains were used. No significant differences between the two strains were noted and the data were pooled. The animals were considered as two groups, those weighing 140-200 Gm. in one (younger, 90 rats), those weighing over 350 Gm. in the other (older, 94 rats). In the latter group most of the rats actually weighed over 400 Gm. and some over 500. While the exact ages of the older rats were not known, many were stated by the commercial breeder to be about one year old. Both males and females were used. Early in the experiment Purina Dog Chow was fed, later Purina Laboratory Chow. The rats were kept in a room regulated at about 75 F. Operations were performed without sterility and with pentobarbital anesthesia. The normal renal alkaline phosphatase activity was determined on the removed right kidney stripped of its capsule and hilar structures, weighed on a Roller-Smith balance, and homogenized in a glass homogenizer with 9 parts of cold 0.9 per cent sodium chloride solution, all in a room at 6 C.

In most of the experiments designed to study the effects of renal ischemia, immediately after right nephrectomy the blood supply to the left kidney was interrupted for either 30 or 60 min. by placing a small artery clamp on the renal artery in the earlier experiments and on the renal artery and vein together after the surrounding fat had been cleared away in later ones. The incision was closed with skin clips to the muscle and skin layers, reopened after 30 or 60 min. with the animal still under anesthesia and sutured after the serrefine had been removed and blood had been seen to be flowing into the kidney. In a smaller number of experiments vascular occlusion on the left was similarly produced, but the right kidney was not molested.

Phosphatase activity was determined within 2 or 3 hours on kidney homogenates usually diluted 1:500 (smaller dilutions being used as necessary) using p-nitrophenyl phosphate in a glycine buffer at pH 9.1 to 9.2 as substrate* (1). Kidney protein concentration was also determined on homogenates (10). Enzyme activity was expressed as moles of substrate split per kilogram of wet kidney tissue per hour (wet activity), and as moles per 100 Gm. of kidney protein per hour. The product of the wet activity times the weight of the kidney is referred to as the activity content of the kidney.

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* Buffer, substrate and standard obtainable from Sigma Chemical Company, St. Louis, Missouri.

RESULTS

A. *Comparison of the alkaline phosphatase activities in the two normal kidneys of individual animals.*—In 5 younger females the ratio of wet activity of the right kidney to that of the left kidney was 0.99 ± 0.04, for 7 older rats (6 males, 1 female) it was 1.02 ± 0.02. The respective values for activity content were 0.99 ± 0.14 and 1.04 ± 0.15. These findings indicate that in the absence of unilateral renal disease, injury, or anomaly, the wet activity and activity content of the right kidney may be taken as a measure of the activity simultaneously present in the left kidney.

B. *Mean enzyme activity of normal kidneys.*—1) Ninety younger animals consisting of 56 males and 34 females were compared with 94 older animals of which 89 were males and 5 females. The means and standard deviations for the wet activities and activity contents of the normal right kidneys of the younger and older groups are listed in table 1.

2) The means and standard deviations of the soluble protein concentrations of the kidneys expressed as grams per kilogram of wet tissue were for 89 younger rats 147.0 ± 15.5, for 93 older rats 147.7 ± 14.4, for 55 younger males 147.6 ± 18.3, for 88 older males 147.4 ± 14.8. Smaller standard deviations for protein determinations can probably be obtained with the method used, but we encountered at times small systematic shifts in our standards.

3) It is obvious from 2 above that the activities per gram of protein would compare as do the wet activities in table 1.

TABLE 1. ALKALINE PHOSPHATASE CONCENTRATION AND CONTENT IN NORMAL RIGHT KIDNEYS OF YOUNGER AND OLDER RATS. Means and standard deviations.

Group	No.	Wet Activity (M/Kg./hr.)	P	Activity Content (mM/hr.)	P
All younger	90	2.94±0.82	<0.001*	2.12±0.59	<0.02
All older	94	1.60±0.44		2.33±0.60	
Younger males	56	2.54±0.54	<0.001	1.85±0.37	<0.001*
Older males	89	1.59±0.44		2.34±0.61	
Younger males	56	2.54±0.54	<0.001	1.85±0.37	<0.001*
Younger females	34	3.58±0.78		2.55±0.63	

* After correction for nonhomogeneity of variance.

C. *The effect of unilateral nephrectomy alone on contralateral renal alkaline phosphatase activity.*—After right kidney excision and assay the uninjured left kidney was assayed at 3, 14, or 21 days. The wet activities and total activity contents are given in table 2 and expressed as the means and standard deviations of the values for the left kidney divided by those for the right kidney for each animal. There seems to be significant difference between the younger and older groups with respect to change in enzyme concentration, but not with respect to enzyme content change. The reduction in enzyme concentration seen in the younger group at 14 and 21 days is caused by a more rapid rate of rise in total left kidney tissue than in specific enzyme substance. Change in kidney weight was related both to change in total body weight (growth) and to increase due to compensatory hypertrophy. At 14 days after nephrectomy the mean change in body weight was plus 22.2 per cent and minus 1.8 per cent for the younger and older groups, respectively, while the left kidney weights exceeded right kidney weights by an average of 49.4 and 15.2 per cent, respectively. Since kidney weight approximately parallels body weight in normal animals in the ranges under consideration (11), the weight increases of the left kidneys due to compensatory hypertrophy alone averaged 27.2 and 17.0 per cent for younger and older rats, respectively, and these means are significantly different, with P being less than 0.02. This calculation neglects the individual differences that may have existed between the normal right and left kidneys before unilateral nephrectomy.

D. *The effect of renal vascular occlusion and concurrent contralateral nephrectomy on renal alkaline phosphatase activity.*—After 30 or 60 min. of circulatory arrest the surface of the kidney is a dark purple. After removal of the serrefine the kidney surface rapidly becomes a lighter red. Edema of the kidney ensues as revealed by a lowering in protein concentration present 3 days after occlusion for 30 min. to a mean of 90 and 81 per cent of the uninjured companion kidney in 6 younger and 6 older rats, respectively. When the right kidney is removed at the same sitting occlusion of the left renal pedicle for 30 min. is followed uniformly by survival in both groups. With occlusion for 60 min. some deaths occur in both younger and older animals without marked difference be-

TABLE 2. ALKALINE PHOSPHATASE CONCENTRATION AND CONTENT IN NORMAL LEFT KIDNEYS FOLLOWING RIGHT NEPHRECTOMY IN THE RAT. Means and standard deviations.

Post Nephrectomy	Group	Number, Sex	Wet Activity	P	Activity Content	P
Days			L/R		L/R	
3	Y	4 (2M, 2F)	1.14 ± 0.19	> 0.1	1.29 ± 0.20	> 0.05
3	O	6M	1.00 ± 0.09		1.09 ± 0.14	
14	Y	10 (5M, 5F)	0.88 ± 0.16	< 0.02	1.30 ± 0.18	> 0.05
14	O	10 (7M, 3F)	1.06 ± 0.12		1.20 ± 0.17	
21	Y	5M	0.81 ± 0.15	= 0.05	1.37 ± 0.23	> 0.05
21	O	6M	1.00 ± 0.13		1.15 ± 0.15	

Y = younger, O = older.

tween the groups. Table 3 summarizes the data for the survivors, expressed as the means and standard deviations of the values for the injured left kidney divided by those for the control right kidney previously removed. The values in table 3 are uncorrected for any changes expected to result from nephrectomy alone. The great variability of the response to ischemia is reflected in the standard deviations. The apparently significant difference between the two groups in wet activity levels at 3 days falls below the 5 per cent level for significance when the activities are expressed in terms of protein content. Because of the great variability of the response to ischemia even large differences between means do not attain significance (viz., activity content at 21 days after 60 min. occlusion). The only difference in means above the horizontal line in table 3 which attains statistical significance is that for the activity content at 14 days after a 30 min. occlusion. Because the groups here are larger than those in the other categories and because P is equal to 0.01 these data would seem to be reliable in indicating a greater enzyme content rise in the younger group. When these figures are compared with those in table 2, the means for the activity content are seen to be 15.1 and 22.1 per cent lower than those present after nephrectomy alone for the younger and older groups, respectively, and a "t" test applied to these differences of the means yields a P value of > 0.05 in the case of the younger group and of < 0.01 for the older group. This means

TABLE 3. ALKALINE PHOSPHATASE CONCENTRATION AND CONTENT IN LEFT KIDNEYS AFTER ISCHEMIA.

Post Injury	Group	Number Sex	Occlusion Time	Wet Activity	P	Activity per Gm. of Protein	P	Activity Content	P
(Days)			(Min.)	L/R		L/R		L/R	
3*	Younger	6 (4M, 2F)	30	0.69 ± 0.26	< 0.05	0.76 ± 0.28	> 0.05‡	0.80 ± 0.29	> 0.05
3*	Older	6M	30	0.41 ± 0.11		0.50 ± 0.09		0.53 ± 0.10	
14*	Younger	16 (12M, 4F)	30	0.72 ± 0.11	> 0.05	.....		1.15 ± 0.21	= 0.01
14*	Older	16M	30	0.81 ± 0.16		.....		0.98 ± 0.14	
14*	Younger	7 (5M, 2F)	60	0.74 ± 0.22	> 0.05	.....		1.03 ± 0.17	> 0.05
14*	Older	4M	60	0.82 ± 0.20		.....		1.23 ± 0.43	
21*	Younger	5 (2M, 4F)	60	0.80 ± 0.28	> 0.05	.....		1.62 ± 0.82	> 0.05
21*	Older	5M	60	0.57 ± 0.26		.....		0.88 ± 0.29	
14†	Younger	6M	60	0.56 ± 0.12	< 0.01	.....		0.51 ± 0.11	< 0.01
14†	Older	6M	60	0.30 ± 0.14		.....		0.25 ± 0.11	

* Right nephrectomy at time of left renal vascular occlusion.
† Right kidney not molested at time of left kidney ischemia.
‡ After correction for nonhomogeneity of variance.

that by the fourteenth day after vascular occlusion the lowering of the enzyme content below the level anticipated as a result of contralateral nephectomy alone is statistically significant for the older group, but not for the younger. The wet activities at 14 days after 30 min. occlusion are significantly lower than those for the nephrectomy controls (table 2), with $P < 0.01$ and < 0.001 for the younger and older groups, respectively.

E. *Comparison of the alkaline phosphatase activities in the two kidneys after unilateral vascular occlusion alone.*—In 6 animals of each group the circulation to the left kidney was interrupted for 60 min. while the right kidney remained unmolested. Both kidneys were assayed 14 days later, and the results are summarized in table 3 below the horizontal line. The L/R values for wet activity are lower than in the groups listed above the horizontal line due mainly to a greater fall in the enzyme concentration of the injured (left) kidney. This is indicative of inhibition of recovery of the injured kidney due to the presence of the uninjured kidney. The activity content ratios are also low compared to those above the line. Between the younger and older groups significant differences are present in the uncorrected data. The wet activity of the kidneys at the time of injury is unknown, but since that of the uninjured kidney of the younger animals might be expected to show a fall (although not as great as after nephrectomy, table 2) the difference between the groups in the absolute falls in wet activity on the injured side is probably smaller than in the ratios in table 3. However, evidence that a difference between the behavior of the two groups does exist is seen in the data for activity content. Since the activity content in the uninjured kidneys of younger rats would be expected to rise at least as much as in the older rats (table 2) the depletion of enzyme activity on the injured side must be less in the younger group than in the older with a probability of greater than 99 per cent that a significant difference exists between the means.

DISCUSSION

As has been stated, the physiologic significance of the enzyme's presence is unknown. The enzyme concentrations and contents in the two normal kidneys of the same animal were about the same. We did not observe the great differences reported by others (4). Our older rats had distinctly lower enzyme concentrations than did our younger rats and within the younger group, females had higher levels than did males. The renal enzyme contents were greater, however, in older males than in younger males because of the greater kidney weights. Because older rats have been reported to develop pyelonephritis, kidneys from both groups were examined microscopically. Significant pyelonephritis or other pathologic conditions sufficient to account for the great differences in enzyme activities of the particular kidneys were not found.

Our experiments on injury are essentially screening experiments using groups containing small numbers of animals. The fall in enzyme concentration seen after unilateral nephrectomy alone in the younger group, but not in the older, is due to the more rapid increase in kidney weight in the younger animals. Total renal enzyme content rose in both groups, but without significant difference between the two. Our finding that compensatory hypertrophy is greater in the younger animals than the older is in agreement with previous work (12).

In the studies in which vascular occlusion was accompanied by immediate contralateral nephrectomy the variability of the response made the detection of possible differences between the groups difficult. There is indication that by 2 weeks after vascular occlusion for 30 min. the enzyme activity content in the older rats had not been regenerated as well toward the level of the expected nephrectomy control as in the younger animals. In view of the variability of the results summarized in table 3 this finding requires confirmation before it can be fully accepted. In the present study the possible influence of sex on the results has not been adequately controlled.

In rats subjected to unilateral occlusion alone with assay of both kidneys deferred for 14 days the injured kidney seemed to be more severely depleted in older rats than in younger. To what extent this represents either greater resistance to ischemia or more rapid recovery from an ischemic injury in the younger has not been determined. Since the groups compared consisted of only 6 rats each the use of a larger number of animals to confirm the difference described would be justified.

Senile rats of definitely known age were not available to us. The use of such rats in studies designed to confirm those findings which seem

to indicate the existence of significant differences between our two age groups would enhance the value of future studies.

SUMMARY

A group (younger) of 90 rats weighing 140 to 200 Gm. was compared with respect to the renal alkaline phosphatase concentration and total alkaline phosphatase content of the kidney with a group (older) of 94 rats with weights of over 350 Gm. Significantly higher enzyme concentrations and lower enzyme contents were present in the total younger group as compared with the older and in the younger males as compared with the older males. Younger females had both higher concentrations and contents as compared with younger males. In the younger group the enzyme concentration of the remaining normal left kidney fell after right nephrectomy and the normal left kidney hypertrophied at a faster rate than in the older in which the enzyme concentration did not fall. The rate of increase in enzyme content did not suffer in the two groups. After clamping of the left renal vessels for 30 min. and concurrent right nephrectomy a drop in enzyme concentration and content in the left kidney was present in both young and old at 3 days with subsequent rise in content. The responses were highly variable with some of the data suggesting that younger rats resist or recover from the effects of ischemia better than do older rats. The same statement is true for the situation obtaining after left renal ischemia when the right kidney was not molested.

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THE HEXOKINASE AND PHOSPHOGLUCOISOMERASE ACTIVITIES OF AORTIC AND PULMONARY ARTERY TISSUE IN INDIVIDUALS OF VARIOUS AGES*

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The present study was undertaken with the purpose of determining the hexokinase and phosphoglucoisomerase activities of homogenates prepared from the aorta and pulmonary artery from individuals of various ages. In the case of the aortic samples the enzyme measurements were carried out both on apparently normal tissue portions and on arterial segments which were the site of arteriosclerotic changes.

METHODS

Seventy samples of the human aorta (thoracic descending) and 68 samples of the pulmonary artery were obtained fresh at autopsy at the St. Louis City Morgue.† The age of the individuals from whom the samples were derived ranged between 0.2 and 83 years. Immediately after removal the samples were placed in sterile beakers immersed in ice.

After separation of the adventitia the percentage areas of the aortic samples which were the site of arteriosclerosis were measured by placing the opened vessels on a glass plate and tracing the normal and arteriosclerotic areas on a piece of transparent paper; the size of these areas was subsequently determined by means of a planimeter. For enzyme activity measurements intima-media samples of normal and arteriosclerotic segments of the aorta and of the pulmonary artery were homogenized in a Pyrex grinder with 9 volumes of distilled water, making a 10 per cent homogenate; the homogenizer was immersed in ice water during the homogenization. For phosphoglucoisomerase determinations the homogenates were diluted with an equal volume of water, making a 5 per cent homogenate.

The hexokinase activity of the arterial homogenates was determined by measuring the rate of disappearance of glucose at 38 C. in a buffer solution containing added ATP, Mg^{++}, KCl, and fluoride.

Two ml. of glucose buffer solution (glucose, 0.0025 M; $MgCl_2$, 0.0025 M; K_2HPO_4, 0.01 M; NaF, 0.025 M; KCl, 0.077 M; tris(hydroxymethyl)aminomethane, 0.03 M, pH 8.0), 0.1 ml. of 0.18 M ATP solution, and 0.9 ml. of water were pipetted into a test tube. The reagent solutions were stored frozen between use. The tube was preheated in a water bath at 38 C. for 5 minutes. At the start of the experiment 2.0 ml. of 10 per cent homogenate were added. A 1.0 ml. aliquot of the homogenate-buffer substrate mixture was removed immediately for glucose analysis, and another aliquot after 30 minutes' incubation.

For precipitation of protein from the removed samples the barium hydroxide-zinc sulfate technique of Somogyi (10) was used; this procedure also serves to stop the enzyme activity of the samples and to eliminate glucose-6-phosphate as a reducing substance in the subsequent glucose analysis (3). The 1.0 ml. aliquots were transferred to test tubes containing 2.0 ml. of 0.3 N barium hydroxide, after which 2.0 ml. of 5 per cent zinc sulfate solution ($ZnSO_4$, 7 H_2O) were added. The contents of the tube were mixed and the tubes allowed to stand for 10 minutes to complete the protein precipitation. The samples were then centrifuged for 10 minutes at 3000 r.p.m. The glucose determinations were performed on aliquots of the supernatants by the method of Nelson (7), employing the reagents described by Somogyi (11). The measurements were carried out at 750 millimicrons, using cuvettes with a 25 mm. light path.

A 30-minute period was chosen for the hexokinase experiments because a decrease in the enzyme activity often was found to take place after this time of incubation. Thus the hexokinase activity observed in a second 30-minute period averaged 77 per cent of that found in the first 30-minute period. A satisfactory degree of correlation was noted between the quantity of

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tissue present in the incubated sample and the amount of glucose utilized.

The phosphoglucoisomerase activity of the arterial homogenates was determined by measuring the rate of formation of fructose-6-phosphate from glucose-6-phosphate at 38 C. in a buffered medium.

Three hundred cubic millimeters of a sodium glucose-6-phosphate solution (0.1 M, pH 7.9), 200 cu.mm. of 1.12 M tris(hydroxymethyl)-aminomethane buffer, pH 7.9, and 1.70 ml. of distilled water were pipetted into a test tube. Constriction pipettes were employed for measuring out the reagents, which were stored frozen between use. The tube was preheated in a water bath at 38 C. for 5 minutes. At the start of the experiment 200 cu.mm. of 5 per cent homogenate were added. A 1.0 ml. aliquot of the homogenate-buffer substrate was removed for fructose-6-phosphate determination immediately after the addition of the homogenate and after 10 minutes' incubation. The incubation period was controlled by means of a stop watch.

The aliquots removed from the homogenate-buffer substrate mixture were rapidly transferred to test tubes containing 5.0 ml. of a 3.3 per cent trichloroacetic acid solution. The trichloroacetic acid served to stop the enzyme activity and to precipitate the protein. The tubes were centrifuged for 10 minutes at 3000 r.p.m., after which the supernatants were filtered through Whatman No. 40 filter paper. 2.0 ml. aliquots of the filtrates were used for fructose determination by the colorimetric method of Roe, Epstein, and Goldstein (8). A reagent blank and a fructose standard were run with each set of analyses.

Experiments were conducted in which the color development of a fructose standard was compared with that of a solution of fructose-6-phosphate. It was found that the color produced by fructose in the form of fructose-6-phate under the conditions of the experiments was 58.5 per cent of that produced by the free fructose. This finding is in good agreement with the values of 55 per cent and 60.5 per cent reported, respectively, by Bruns and Hinsberg (2) and by LePage (6). A factor of 2.46 was therefore employed to convert the fructose readings obtained in analyses of the filtrate samples to fructose-6-phosphate.

A high degree of proportionality was observed between time and the quantity of fructose-6-phosphate formed by the tissue over the first 10 minutes of incubation; thus the values obtained after 5 minutes of incubation averaged 47 per cent of the 10-minute values. The relation between the amount of tissue in the sample and the quantity of fructose-6-phosphate formed was evaluated by comparison of the values found in experiments with 100 and 200 cu.mm. of homogenate of the same arterial samples. The phosphoglucoisomerase values for the 100 cu.mm. homogenate samples averaged 54 per cent of those for the 200 cu.mm. samples.

In the case of both the aortic and pulmonary artery samples determinations of the dry weight, total nitrogen, and cholesterol content of the tissue were made on a portion of the arterial wall adjacent to that employed for the enzyme studies. The nitrogen analyses were carried out by macro-Kjeldahl procedure. For the cholesterol determinations the procedure of Knobil and associates (5) was used.

RESULTS

The results of the hexokinase and phosphoglucoisomerase determinations on the individual aortic and pulmonary artery samples, and the mean values recorded for the various decades have been listed in table 1. The table further contains the observed dry weight, total nitrogen, and cholesterol values for the samples. In addition, the percentage values of the areas of the aortic samples which were the site of arteriosclerosis have been included.

Aortic Samples

I. *Normal Aortic Tissue*

Hexokinase—The average quantity of glucose metabolized by the non-arteriosclerotic tissue samples (N=63) was 0.010 mM (1.8 mg.)/Gm. wet tissue/hour, and 0.27 mM (46.6 mg.)/Gm. tissue nitrogen/hour. It will be seen from the mean values recorded for the various decades that the hexokinase activity of the samples obtained from children aged 0.2-4 years was lower than the activity of samples derived from adults. This difference is most apparent when the values expressed on the basis of the tissue nitrogen content are considered (t of difference between 0.2-4 year samples and 10-83 year samples, 3.24).

A calculation of the coefficient of correlation between age and hexokinase activity of the samples from the 10-83 year subjects failed to show any significant variation in enzyme activity with age:

TABLE 1. HEXOKINASE AND PHOSPHOGLUCOISOMERASE ACTIVITIES OF 70 HUMAN AORTIC SAMPLES AND 68 PULMONARY ARTERY SAMPLES

No.	Age (Years)	Sex, Race	NORMAL AORTIC TISSUE							ARTERIOSCLEROTIC AORTIC TISSUE								PULMONARY ARTERY TISSUE						
			Dry Matter, %	Nitrogen, % of Wet Weight	Cholesterol, % of Wet Weight	Glucose Metabolized, mM/Gm. Wet Tissue/Hour	Glucose Metabolized, mM/Gm. Tissue N/Hour	F-6-P Formed, mM/Gm. Wet Tissue/Hour	F-6-P Formed, mM/Gm. Tissue N/Hour	Dry Matter, %	Nitrogen, % of Wet Weight	Cholesterol, % of Wet Weight	Glucose Metabolized, mM/Gm. Wet Tissue/Hour	Glucose Metabolized, mM/Gm. Tissue N/Hour	F-6-P Formed, mM/Gm. Wet Tissue/Hour	F-6-P Formed, mM/Gm. Tissue N/Hour	Area of Arteriosclerotic Tissue, in Percentage	Dry Matter, %	Nitrogen, % of Wet Weight	Cholesterol, % of Wet Weight	Glucose Metabolized, mM/Gm. Wet Tissue/Hour	Glucose Metabolized, mM/Gm. Tissue N/Hour	F-6-P Formed, mM/Gm. Wet Tissue/Hour	F-6-P Formed, mM/Gm. Tissue N/Hour
1	0.2	F C	25.0		0.2	0.013		1.28														1.04	
2	0.5	M C	34.7	4.98	0.2	0.009	0.18	2.02	41									29.4	3.98	0.2			1.69	43
3	0.6	F C	31.5	4.50	0.2	0.003	0.07	0.82	18									34.8	3.22	0.2	0.003	0.09	1.25	39
4	1	F W	32.6	5.63	0.2	0.022	0.39	1.72	31									36.7	5.16	0.2			1.96	38
5	2	M C	36.9	4.88	0.2	0.009	0.19	1.37	28											0.3			1.79	
6	2	M C	30.9	4.65	0.3	0.007	0.15	1.31	28									31.2	4.35	0.2	0.018	0.41	0.93	21
7																		22.9	3.46	0.1			0.61	18
8	4	M W	33.3	5.12	0.1	0.011	0.21	1.66	32															
9	4	M C	36.5	5.47	0.2	0.003	0.06	1.27	23												0.003		0.80	
10	4	M C	34.8	5.33	0.2	0.005	0.09	1.30	24									31.4	4.65	0.2	0.003	0.07	1.07	23
	Mean.....		34.1	5.06	0.2	0.009	0.17	1.42	28									31.1	4.15	0.2	0.007	0.19	1.24	30
11	10	M C	33.4	4.66	0.2	0.005	0.11	1.20	26									31.4	4.65	0.2	0.003	0.07	1.22	26
12	14	M C	31.3	4.04	0.2	0.010	0.25	1.62	40									24.5	3.11	0.2	0.011	0.35	1.81	58
13	17	M W	28.4	3.99	0.4	0.012	0.30	2.06	52	29.7	4.05	0.4	0.014	0.35	1.81	45	12	26.5	3.59	0.3	0.013	0.36	2.21	62
	Mean.....		31.0	4.23	0.3	0.009	0.22	1.62	39	29.7	4.05	0.4	0.014	0.35	1.81	45		27.5	3.88	0.2	0.009	0.26	1.75	49
14	21	M C	33.3	3.50	0.3	0.013	0.37	1.85	53									36.9	3.42	0.3	0.003	0.09	1.82	53
15	23	F W	33.2	4.02	0.4	0.014	0.35	1.21	30	29.6	3.76	0.5			1.36	36	13	29.1	3.90	0.4	0.017	0.44	2.29	59
	Mean.....		33.3	3.76	0.4	0.014	0.36	1.53	42	29.6	3.76	0.5			1.36	36		33.0	3.66	0.4	0.010	0.27	2.06	56
16	33	M C	34.1	3.79	0.4	0.007	0.19	1.56	41								1	22.6	2.93	0.2			0.85	29
17	35	F W	32.5	4.39	0.4	0.014	0.32	1.82	41									27.6	2.64	0.3	0.012	0.47	2.24	85
18	35	F W	28.9	3.83	0.6	0.018	0.47	3.06	80	27.4	3.51	0.6	0.018	0.51	2.31	66	39	29.2	4.01	0.4	0.018	0.45	3.10	77
19	35	F C	32.5	4.64	0.3	0.008	0.17	1.44	31								1	25.9	3.59	0.1			1.37	38
20	36	M C	30.3	4.42	0.6	0.015	0.34	1.78	40				0.010		1.71		2	27.2	4.08	0.2	0.008	0.20	1.10	27
21	37	F C	27.3	3.53	0.7	0.012	0.34	1.66	47	26.8	3.29	1.2	0.005	0.15	1.46	44	11	23.8	3.34	0.3	0.009	0.26	1.14	34
22	38	M C	30.7	4.17	0.5	0.014	0.34	1.70	41	32.7	4.30	0.5	0.017	0.40	2.07	48	6							
23	39	F W	27.2	3.60	0.2	0.018	0.50	1.82	51									23.9	3.35	0.2	0.014	0.42	1.50	45
	Mean.....		30.7	4.04	0.5	0.013	0.33	1.85	47	28.9	3.70	0.8	0.013	0.35	1.88	53		25.7	3.34	0.3	0.012	0.36	1.62	48
24	42	M W								32.2	4.32	0.7	0.018	0.42	1.03	24	100	32.5	3.56	0.6	0.015	0.42	1.39	39
25	44	M W	33.8	4.60	0.6	0.006	0.13					0.5			1.12	22	17							
26	44	M C	26.6	3.24	0.5	0.008	0.25	1.28	39	27.1	3.19	0.5	0.009	0.28	1.47	46	70	26.5	3.23	0.3	0.011	0.34	1.25	39
27	46	M W						1.55		28.9	3.44		0.007	0.20			95	27.8	3.71	0.3	0.004	0.11	0.89	24
28	46	F W	29.7	3.83	0.6	0.012	0.31	1.90	50	28.5	3.62	0.8	0.011	0.30	1.74	48	95	25.2	3.07	0.4	0.013	0.42	2.48	81
29	46	F C	31.7	4.16	0.5	0.009	0.22	1.81	43	38.6	4.15	1.4	0.003	0.07	1.70	41	50	26.1	3.54	0.3	0.006	0.17	1.50	42
30	46	F C	39.9	4.44	0.8	0.007	0.16	1.64	37	40.0	4.03	0.9	0.007	0.17	1.80	45	1	30.6	4.41	0.3	0.006	0.14	1.79	41
31	48	M W	28.6	3.85	0.5	0.008	0.21	2.15	56	29.4	2.83	1.2	0.010	0.35	1.99	70	27	24.6	3.41	0.4	0.009	0.26		
32	49	F W	27.7	3.32	0.7	0.013	0.39	2.09	63									31.7	3.21	0.3	0.011	0.34	1.18	37
	Mean.....		31.2	3.90	0.6	0.009	0.24	1.78	48	32.1	3.68	0.9	0.009	0.26	1.55	42		28.1	3.51	0.4	0.009	0.28	1.50	43
33	50	M W	24.3	3.05	0.8	0.009	0.29	1.39	46	25.5	2.97	0.9	0.009	0.30	1.18	40	24	24.5	3.10	0.3	0.013	0.42	1.98	64
34	50	M W	29.8	3.43	0.6	0.010	0.29	1.89	55	26.5	2.71	1.2	0.012	0.44	2.28	84	43	30.7	3.72	0.4	0.008	0.22	1.81	49
35	50	F C	26.3	3.42	0.5	0.012	0.35	1.78	52	26.3	3.42	0.7	0.011	0.32	1.56	46	17	25.1	3.42	0.5	0.017	0.50	1.66	49
36	52	M C	30.3	4.05	0.2	0.010	0.25	1.75	43	30.2	4.13	1.2	0.012	0.29	1.50	36	37	24.9	2.85	0.4	0.015	0.53	2.56	90
37	53	M W	22.7	2.71	0.4	0.008	0.30	1.04	38	39.0	2.87	1.7	0.004	0.14	0.77	27	22	34.6	4.00	0.7			1.25	31
38	53	M C	29.7	4.18	0.7	0.009	0.22	1.97	47	31.6	3.77	1.5	0.008	0.21	1.95	52	15	27.6	3.93	0.4	0.009	0.23	1.33	34
39	54	M C	27.2	3.43	0.6	0.014	0.41	1.48	43	25.2	3.08	0.5	0.009	0.29	0.92	30	31	23.5	3.31	0.4	0.010	0.30	1.54	46
40	54	F W	29.2	2.83	0.7	0.006	0.21	1.53	54	29.2	3.54	2.8	0.007	0.20	1.03	29	28	23.3	3.07	0.6	0.007	0.23	1.42	46
41	54	F C	25.0	2.94	0.6	0.009	0.31	1.58	54	23.7	2.27	3.2	0.009	0.40	1.12	49	18	25.1	3.17	0.4	0.008	0.25	1.22	38
42	55	M W	33.7	4.48	0.5	0.011	0.25	1.22	27	32.4	3.95	1.2	0.007	0.18	1.13	29	19	39.5	5.55	0.5	0.004	0.07	1.80	32

TABLE 1 (Continued)

No.	Age (Years)	Sex, Race	NORMAL AORTIC TISSUE							ARTERIOSCLEROTIC AORTIC TISSUE								PULMONARY ARTERY TISSUE						
			Dry Matter, %	Nitrogen, % of Wet Weight	Cholesterol, % of Wet Weight	Glucose Metabolized, mM/Gm. Wet Tissue/Hour	Glucose Metabolized, mM/Gm. Tissue N/Hour	F-6-P Formed, mM/Gm. Wet Tissue/Hour	F-6-P Formed, mM/Gm. Tissue N/Hour	Dry Matter, %	Nitrogen, % of Wet Weight	Cholesterol, % of Wet Weight	Glucose Metabolized, mM/Gm. Wet Tissue/Hour	Glucose Metabolized, mM/Gm. Tissue N/Hour	F-6-P Formed, mM/Gm. Wet Tissue/Hour	F-6-P Formed, mM/Gm. Tissue N/Hour	Area of Arteriosclerotic Tissue, in Percentage	Dry Matter, %	Nitrogen, % of Wet Weight	Cholesterol, % of Wet Weight	Glucose Metabolized, mM/Gm. Wet Tissue/Hour	Glucose Metabolized, mM/Gm. Tissue N/Hour	F-6-P Formed, mM/Gm. Wet Tissue/Hour	F-6-P Formed, mM/Gm. Tissue N/Hour
43	55	M W	25.8	3.13	0.6	0.014	0.45	1.90	61	27.6	3.19	1.5	0.014	0.44	1.81	57	17	21.0	2.53	0.3	0.013	0.51	1.73	68
44	56	M W	30.0	3.93	0.8	0.010	0.26	1.81	46	29.7	3.74	0.6	0.007	0.19	1.76	47	19	28.3	3.30	0.3	0.007	0.21	1.19	36
45	57	M W								31.5	4.10	0.5	0.003	0.07	0.92	23	70	32.7	4.50	0.3	0.011	0.25	1.11	25
46	57	M W								29.3	2.84	1.7	0.003	0.11	0.98	34	78	19.3	2.61	0.4	0.004	0.15	1.23	47
47	57	F W	27.7	3.62	0.6	0.008	0.22	1.53	42	27.7	3.11	0.8	0.005	0.16	0.45	15	8	27.5	3.94	0.2	0.008	0.20	1.69	43
48	59	M W			0.7			1.69		27.8	3.60	4.5	0.012	0.33	0.84	23	47	27.2	3.73	0.3	0.007	0.19	1.22	33
49	59	M W	28.5	2.98	1.3	0.013	0.44	1.93	65	39.2	1.16	2.1	0.011	0.94	1.80	129	24	24.8	3.04	0.5	0.013	0.43	1.93	63
	Mean		27.9	3.44	0.7	0.010	0.30	1.63	45	29.6	3.20	1.6	0.008	0.20	1.28	44		27.0	3.52	0.4	0.010	0.29	1.57	47
50	60	M W	28.8	3.91	0.5	0.006	0.15	1.22	31	23.9	3.09	1.8	0.004	0.13	0.82	27	36	24.0	3.18	0.5	0.013	0.41	1.12	35
51	60	M W	25.9	3.19	0.7	0.019	0.60	2.16	68	25.6	2.82	2.9	0.017	0.58	1.85	65	93	19.3	2.61	0.5	0.016	0.61	1.96	75
52	60	F W	28.5	3.29	1.2	0.008	0.24	1.00	30	44.6	2.23	3.3	0.000	0.00	1.01	45	23	27.3	3.60	1.3	0.006	0.17	0.39	11
53	61	M W	28.6	3.80	0.4	0.013	0.34	1.63	43	33.2	4.11	2.9	0.009	0.22	1.27	31	32	23.3	3.17	0.3	0.009	0.29	1.24	39
54	61	M W	30.8	3.82	0.9	0.008	0.21	1.71	45	26.3	1.77	1.5	0.012	0.68	1.23	70	50	25.3	3.26	0.6	0.013	0.40	2.00	61
55	62	M W	29.0	3.71	0.8	0.007	0.19	1.95	72	26.5	3.25	2.2	0.004	0.12	1.68	52	14	25.9	3.67	0.8	0.007	0.19	1.81	50
56	63	M W	28.5	3.87	0.7	0.007	0.18	1.43	45	29.8	3.34	1.5	0.009	0.27	1.12	33	5	26.7	3.75	0.5	0.008	0.21	0.77	21
57	63	M W	30.7	3.81	1.2	0.008	0.21	1.31	34	32.8	5.43	2.1	0.007	0.13	0.77	14	98	25.8	3.52	1.1	0.009	0.26	1.40	40
58	63	M W	24.0	3.03	0.6	0.010	0.33	1.59	52	31.0	2.43	0.7	0.007	0.29	1.18	49	72	25.2	3.19	0.5	0.012	0.38	1.55	49
59	64	M W	25.5	3.48	0.5	0.008	0.23	1.25	36	28.6	2.80	2.9	0.007	0.25	1.18	42	19	22.7	3.31	0.3	0.011	0.33	1.57	47
60	64	M W								31.7	3.34	0.6	0.014	0.42	1.28	38	100	21.0	2.85	0.4	0.020	0.70	1.84	64
61	64	M W	29.2	3.89	0.9	0.003	0.08	1.00	41	33.1	3.63	4.0	0.003	0.08	1.14	31	39	27.0	3.77	0.9	0.005	0.13	1.00	26
62	65	M W								27.8	3.71	1.4	0.016	0.43	2.02	54	100	21.3	2.93	0.3	0.015	0.51	2.04	69
63	65	M C	25.6	3.36	0.5	0.006	0.18	1.43	43	27.6	3.56	0.7	0.006	0.17	2.06	58	10	26.1	3.62	0.3	0.008	0.22	1.71	47
64	66	M W	30.7	3.49	1.6	0.008	0.23	1.83	52	42.1	1.88	3.8	0.004	0.21	1.49	70	36	24.3	3.08	0.4	0.018	0.58	1.59	52
65	69	M W	30.1	3.92	0.7	0.012	0.31	2.22	57	29.6	3.76	3.1	0.007	0.19	1.71	46	17	29.1	3.90	0.5	0.012	0.31	1.47	38
66	69	M W	29.3	3.82	0.9	0.011	0.29	2.13	56	33.0	3.40	1.4	0.009	0.26	1.30	38	42	24.1	3.10	0.5	0.003	0.10	1.85	60
67	69	M W	26.3	3.68	0.7	0.004	0.11	1.52	41	29.3	4.09	0.6	0.003	0.07	0.96	24	29	29.2	4.18	0.3	0.004	0.10	1.32	31
	Mean		28.2	3.64	0.8	0.009	0.24	1.63	47	31.4	3.26	2.1	0.008	0.25	1.34	44		24.9	3.37	0.6	0.011	0.33	1.48	45
68	71	M W	29.2	3.43	0.7	0.011	0.32	1.50	44	30.7	3.01	5.9	0.007	0.23	1.20	40	15	25.2	3.16	0.5	0.011	0.35	1.20	38
69	72	M W	30.4	3.95	1.4	0.008	0.20	1.23	31	33.4	2.92	5.5	0.006	0.20	1.11	38	38	23.0	3.02	1.7	0.014	0.46	1.86	62
70	78	F C	32.2	2.71	0.9	0.018	0.67	1.26	47	36.3	4.20	8.3	0.006	0.14	1.55	37	35	21.9	2.77	0.8	0.014	0.50	1.58	57
71	83	M W	28.9	3.53	0.8	0.006	0.17	1.27	36	32.4	4.46	1.2	0.005	0.11	1.39	31	42	23.2	3.19	0.2	0.006	0.19	1.03	32
	Mean		30.2	3.40	1.0	0.011	0.34	1.32	40	33.2	3.64	5.2	0.006	0.17	1.31	37		23.3	3.03	0.8	0.011	0.38	1.42	47
Grand Mean			29.8	3.88	0.6	0.010	0.27	1.62	44	30.6	3.32	1.9	0.0085	0.27	1.39	43		26.9	3.52	0.4	0.010	0.31	1.51	45

Age/quantity of glucose metabolized/Gm. wet tissue:

$r = -0.20$ ($t = 1.47$, $N = 54$)

Age/quantity of glucose metabolized/Gm. tissue nitrogen:

$r = -0.02$ ($t = 0.14$, $N = 54$)

An analysis of the hexokinase values recorded for the two sexes of this group of subjects revealed that the mean enzyme activity of the samples derived from the women was 122 per cent of the values observed for the men, when expressed on the basis of wet tissue weight, and 125 per cent, when calculated on the basis of the tissue nitrogen content.

Phosphoglucoisomerase—The mean quantity of fructose-6-phosphate formed from glucose-6-phosphate by the 64 samples investigated was 1.62 mM (421 mg.)/Gm. wet tissue/hour, and 44 mM (11.4 Gm.)/Gm. tissue nitrogen/hour. As in the case of the hexokinase determinations the phosphoglucoisomerase values of the samples from children aged 0.2-4 years were significantly lower than the values observed for aortic tissue from adults when calculated on the basis

TABLE 2. MEAN ENZYME ACTIVITIES OF ARTERIOSCLEROTIC TISSUE EXPRESSED IN PERCENTAGE OF ACTIVITIES OF NORMAL TISSUE OF THE SAME AORTIC SAMPLES

Age Group (Years)	Hexokinase						Phosphoglucoisomerase					
	Wet Tissue			Tissue Nitrogen			Wet Tissue			Tissue Nitrogen		
	N	%	t of diff.	N	%	t of diff.	N	%	t of diff.	N	%	t of diff.
30-39	4	82	0.99	3	90	0.37	4	95	0.73	3	98	0.55
40-49	5	92	0.99	5	102	0.09	5	100	0.22	5	111	1.62
50-59	14	87	2.12	14	102	0.42	14	80	3.08	14	96	0.25
60-69	16	79	2.73	16	95	0.38	16	82	3.87	16	95	0.45
70-83	4	64	1.92	4	65	1.40	4	100	0.00	4	94	0.84
Average..		81			91			91			99	

of the tissue nitrogen content (t of difference, 5.58). A calculation of the coefficient of correlation between age and phosphoglucoisomerase activity of the samples from the 10-83 year individuals showed no significant change in enzyme activity with age:

Age/quantity of F-6-P formed/Gm. wet tissue:
$r = -0.16$ ($t = 1.18$, $N = 55$)

Age/quantity of F-6-P formed/Gm. tissue nitrogen:
$r = +0.08$ ($t = 0.58$, $N = 53$)

The phosphoglucoisomerase activity of the samples derived from the women of this age group was 102 per cent of that found for the men, both when expressed on the basis of wet tissue weight and tissue nitrogen content.

II. *Arteriosclerotic Aortic Tissue*

Determinations of the hexokinase and phosphoglucoisomerase activities of arteriosclerotic aortic tissue were made on 53 samples; in 43 of these samples sufficient normal arterial tissue was available to permit determinations also on the normal portions of the vessel wall.

The values for the individual aortic samples listed in table 1 do not reveal a regular tendency towards lower enzyme activities in the arteriosclerotic tissue portions as compared with the normal areas; in some instances, however, a considerable reduction in the enzyme values of the arteriosclerotic tissue may be noted. In table 2 the average enzyme values of the 43 arteriosclerotic samples have been recorded for the various decades in percentages of the values found for the normal tissue. It will be seen from the table that significantly lower mean hexokinase and phosphoglucoisomerase values were observed for the 30 samples of the 50-59 and 60-69 year age groups when the enzyme activities were expressed on the basis of wet tissue weight; in contrast to this no significant difference was found when the activities were calculated on the basis of the tissue nitrogen content.

Pulmonary Artery Samples

Both the hexokinase ($N=60$) and phosphoglucoisomerase ($N=67$) values observed for the pulmonary artery tissue were found to be of the same order of magnitude as the values recorded for the aortic samples.

Hexokinase—The average quantity of glucose metabolized by the pulmonary artery tissue was 0.010 mM (1.8 mg.)/Gm. wet tissue/hour, and 0.31 mM (55.8 mg.)/Gm. tissue nitrogen/hour. The average values found for the women of the 10-83 year age group were respectively, 107 and 103 per cent of those recorded for the men.

No significant variation in the enzyme activity with age was noted; for the samples derived from subjects aged 10-83 years the coefficient of correlation Age/hexokinase activity was found to be $+0.01$ ($t = 0.07$, $N = 56$) when calculated on the basis of wet tissue weight, and $+0.08$ ($t = 0.59$, $N = 56$), when expressed on the basis of the tissue nitrogen content.

Phosphoglucoisomerase—The average quantity of fructose-6-phosphate formed from glucose-6-phosphate by the samples of pulmonary artery tissue examined was 1.51mM (393 mg.)/Gm. wet tissue/hour, and 45 mM (11.7 Gm.)/Gm. tissue nitrogen/hour. The mean values found for the samples from the women of the 10-83 year age group were, respectively, 107 and 106 per cent of those observed for the men. The coefficient of correlation Age/phosphoglucoisomerase activity for the samples of this age group was -0.20 ($t = 1.53$, $N = 58$) when expressed on the basis of wet tissue weight, and -0.08 ($t = 0.60$, $N = 58$), when calculated on the basis of the tissue nitrogen content.

DISCUSSION

In a previous study from this laboratory on intact human aortic tissue a mean glucose utilization of 1.5 mg., or 0.0084 mM/Gm. dry tissue/hour was observed (4). These values

correspond to a utilization by the wet tissue of approximately 0.43 mg., or 0.0024 mM of glucose/Gm. wet tissue/hour. In the present investigation in which the experiments were carried out on tissue homogenates instead of intact arterial tissue and supplementation with ATP was used the average glucose utilization recorded was 1.8 mg., or 0.010 mM/Gm. wet tissue/hour.

The enzyme activity values observed in the present study have revealed a phosphoglucoisomerase activity of the arterial tissue which is approximately 150 times greater than the hexokinase activity when expressed on the basis of millimoles of substrate metabolized. The hexokinase values found are about ⅓ of the activity reported for rabbit erythrocytes by Rubinstein, Ottolenghi and Denstedt (9). With regard to the arterial phosphoglucoisomerase activity the values observed in the present investigation are approximately 350 times higher than the activity value of normal human serum (2) and twice the activity of human red blood cells (1).

It is of interest to note that in many instances comparatively little difference was found between the activities of apparently normal areas of the aortic wall and of the sections which were the site of macroscopic arteriosclerosis. The fact that a difference in mean enzyme activities of normal and pathologic tissue was observed for the samples derived from 50-69 year old individuals only when the values were calculated on the basis of wet tissue weight but not when expressed on the basis of the tissue nitrogen content might indicate that the lower values found for the arteriosclerotic tissue per unit of wet weight may be due, at least partly, to a replacement of the arterial tissue by inert, non-nitrogenous, material.

SUMMARY

Determinations were made of the hexokinase and phosphoglucoisomerase activities of homogenates of 70 samples of the human aorta and 68 samples of the pulmonary artery, derived from individuals aged 0.2 to 83 years. In the case of the aortic samples enzyme measurements were carried out both on normal and arteriosclerotic areas of the vessel wall

The hexokinase measurements revealed a mean glucose utilization of 0.010 mM/Gm. wet tissue/hour for both the normal aortic and the pulmonary artery tissue; for the arteriosclerotic aortic tissue the average value was 0.0085 mM/Gm. wet tissue/hour. The corresponding phosphoglucoisomerase values, expressed as mM of F-6-P formed/Gm. wet tissue/hour, were 1.62, 1.51, and 1.39.

No significant variation with age was found in the hexokinase and phosphoglucoisomerase activities of the normal aortic samples and the pulmonary artery samples obtained from subjects aged 10-83 years. Although a notable decrease in the enzyme activity of the arteriosclerotic tissue as compared with the normal tissue was occasionally observed no significant difference was found in the mean enzyme values of the arteriosclerotic and normal tissue portions when the values were calculated on the basis of the tissue nitrogen content.

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THE EFFECT OF SENESCENCE ON RESISTANCE TO STRESS
I. Response of Young and Old Mice to Cold

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It is generally assumed that old organisms do not withstand stress as well as the young. Essentially, this assumption is based on observations where large numbers of humans of different age groups were exposed to the same disastrous or stressful situation. Whitehorn reported that during World War I and during the economic depression of the 1930's psychoses increased in the old but not in the young (35). Similarly, in World War II old people were far less able to withstand the stress of concentration camp life than the young (23). Several hypotheses on the relation between stress and aging have arisen out of similar observations (28). Such observations made on the social scene require experimental verification in the laboratory not only to place them on a more secure basis but, more important, to identify the functional impairment(s) responsible for the defect in the aged organism's ability to sustain stress with the eventual objective of providing the necessary basis of preventative and therapeutic recommendations.

Reports of experimental studies on this subject have appeared in the literature, many of which indicate that age differences in response to stress are not always easily demonstrable in the laboratory. For example, very little difference was found in the rate of wound healing of young and old rats (4). Also, while some drugs were found to be more toxic to old animals than to young, others were less so (6). Krag and Kountz (22) found that the oxygen consumption of elderly subjects in an air temperature of 5 to 15 C., was greater than that of the young, whereas Horvath and associates (21) reported a greater heat production in young persons as compared with old subjects on exposure to an ambient temperature of 10 C. In both studies, however, the old experienced less subjective discomfort than did the young. Obviously, more studies are required in this field.

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METHODS

The investigations to be described here deal with a stress commonly experienced by large numbers of individuals under natural circumstances, namely cold. Under the controlled conditions of the laboratory, this particular stress has the advantage that the animal cannot circumvent it by his instinctive devices, as would be possible for example in fasting where an animal might begin to eat his feces. In addition, much information is available on the physiology of adaptation to cold, and hence there exists a factual basis for further gerontologic study.

Because of the very large numbers of animals required for these studies, a small experimental animal had to be selected. The animal chosen was the mouse, chiefly because of the small difference in size between the mature young and the old in this species. The differences in weight between a 4 month old and 2 year old mouse is about 20 per cent, whereas in rats it is around 200 per cent. Hence, when differences in size as well as in age may play a role in the function under gerontologic study, it is preferable to use mice rather than rats. Furthermore, the C56BL mouse was used in this study because of its well-defined and well-investigated genetic background. Mature, female, non-pregnant mice were used in all experiments, the young being 4 to 7 months old, while the old were 16½ to 21 months. Purina fox chow, biscuit or powder, and water were made available to the animals ad libitum.

Eight experiments were conducted and the following procedure was common to all: there was first a control period of 8 days when the mice were maintained in an air-conditioned warm room at a temperature of 26 to 27 C. Then the animals were moved from the warm room into a "walk-in" cold room, where they were kept for 14 days. The temperature of the cold room was varied in different experiments (table 1). Thereafter, the mice were returned to the original warm room where they were maintained for another 8 days, after which the experiment was terminated.

TABLE 1. DETAILS OF PROCEDURE.

Experiment	Temperature of Cold Room (°C.)	Criteria Studied
1	−1 to +1	Survival, food intake, body weight
2	2 to 4	Survival
3	6 to 7	Survival
4	9 to 11	Survival, B.M.R., food intake, body weight
5	9 to 11	Survival, blood sugar
6	9 to 11	Survival, red blood, white blood, and circulating eosinophil count
7	15 to 17	Survival
8	1.5 to 3.5	Survival, body weight, and food intake (temperature gradually lowered)

Some experiments were conducted with the sole objective of determining the survival and mortality of young and old mice in the cold; in other experiments various physiologic criteria were investigated as well as survival (table 1). These determinations were always carried out at the temperature of the room in which the animals were currently being maintained. The oxygen consumption was determined by a method described earlier (14). The food intake studies made use of special food trays described by Guest, Brodsky, and Nelson (18). An adaptation of Nelson's micro-method was used to determine the blood sugar (25). The red and white blood cells were counted by the usual routine methods in the Spencer "bright-line" haemocytometer using Hayem's and Turk's fluid as diluents. The eosinophil counts were determined in the Fuchs-Rosenthal counting chamber using the Speirs-Meyer diluent (34). At least two chambers were counted for each animal. However, for the initial count before the stress, as many chambers were counted per each animal as yielded a minimum of 24 eosinophils (16). To obtain the circulating lymphocyte and "polymorph" counts, the percentage of these cells were obtained from a count of 200 leucocytes in a blood smear stained with Wright's stain and multiplied by the white blood cell count. Samples of blood were always taken from the tail.

The data obtained in the warm room prior to the exposure to the cold were assigned a value of 100 per cent. In the case of the oxygen consumption, food intake and body weight studies, the value of 100 per cent was assigned to the mean of as many as 6 control readings per animal. However, in the studies involving the taking of blood, only one control reading in the warm room was obtained so as not to damage the tail for subsequent study, and this was assigned the value of 100 per cent. The results of the metabolic and blood studies subsequent to the first warm room values were expressed as a percentage of these "warm room" values.

Three types of statistical analysis were carried out: chi-square tests for determining levels of significance of differences in mortality rate, and t tests and analyses of variance for determining the significance of differences in oxygen consumption, food intake, body weight, blood counts, and blood sugar. A difference was considered significant when $P < 0.05$.

RESULTS

Survival of Young and Old Mice in the Cold: In Experiment 1, 15 young mice were housed in one wooden box, while 16 old mice were maintained together in another. When they were placed for 14 days in the cold room at −1 to +1 C., none of the young died and only 3 of the old succumbed, 2 on the first day and another on the seventh day. There was no significant difference in the mortality rate between young and old animals in this experiment ($0.10 > P > 0.05$).

In all subsequent experiments the mice were housed one animal per metal cage, the bottom of which contained a wooden board, on which

TABLE 2. SURVIVAL OF YOUNG AND OLD C57BL FEMALE MICE EXPOSED FOR 14 DAYS IN THE COLD.

No. of Days in Cold Room	Expt. 2 (2 to 4 C.)		Expt. 3 (6 to 7 C.)		Expts. 4 to 6 (9 to 11 C.)		Expt. 7 (15 to 17 C.)	
	Young Dead	Old Dead	Young Dead	Old Dead	Young Dead	Old Dead	Young Dead	Old Dead
1	25	29	1	17	1	10	0	0
2	3	0	0	5	0	4	0	0
3	1	0	0	0	1	1	0	0
4	1	0	0	0	0	2	0	0
5	0	0	3	0	1	1	0	0
6	0	0	1	0	1	4	0	0
7	0	0	1	0	0	0	0	1
8	0	0	0	0	1	1	0	0
9	0	0	0	0	0	0	0	0
10	0	0	0	1	0	0	0	0
11	0	0	0	0	0	0	0	0
12	0	0	2	1	0	3	0	0
13	0	0	0	1	0	0	0	0
14	0	0	0	0	4	4	0	0
Total number dead.....	30	29	8	25	9	30	0	1
No. of Mice Stressed.....	30	29	30	30	69	69	20	20
Percentage Dead	100	100	27	83	13	43	0	5
Significance of Difference (P).	=1		<0.001		<0.001		0.8> P >0.7	

TABLE 3. SURVIVAL OF YOUNG AND OLD MICE ON GRADUAL EXPOSURE TO ENVIRONMENTAL TEMPERATURE OF 1.5 TO 3.5 C.

Temperature of Cold Room (°C.)	10 to 12			5.5 to 7.5				1.5 to 3.5							Total Number Dead	Number of Mice Stressed
Day of Exposure	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
Deaths—Young	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	20
Old	2	0	0	3	1	1	1	0	2	1	1	3	0	1	16	20

the mice could stand. The survival of the mice in Experiments 2 to 7, inclusive, are given in table 2. The results of this table clearly show that statistically significant age differences in mortality due to cold become apparent only within a certain temperature range. Thus, too low a temperature (2 to 4 C.) killed 100 per cent of both young and old by the fourth day (table 2 Experiment 2). Too high a temperature, on the other hand, (15 to 17 C.) killed 0 per cent of the young and 5 per cent of the old mice (table 2, Experiment 7, $0.8 > P > 0.7$). However, exposing the mice to a temperature of 6 to 7 C. killed 83 per cent of the old mice, but only 27 per cent of the young, a highly significant difference (table 2, Experiment 3, $P < 0.001$). When mice were exposed to a slightly higher temperature of 9 to 11 C. for the same period, then 43 per cent of the old died as against 13 per cent of the young, a highly significant difference (table 2, Experiments 4 to 6, $P < 0.001$). When differences in mortality rate due to age were observed, they were especially marked on the first day of the stress.

Neither young nor old mice were able to survive the stress of an environmental temperature of 2 to 4 C. longer than 4 days when taken directly to it from a room temperature of 26 to 27 C. (table 2). Therefore, Experiment 8 was carried out to study the survival of mice brought to the low temperature more gradually. In this experiment the environmental temperature was first lowered from 26 to 27 C. to 10 to 12 C. and kept at this level for 3 days. As a result, 2 of the 20 old mice died and none of the young (table 3). Thereafter, the temperature was further lowered to 5.5-7.5 C. for 4 days resulting in the death of 6 more old and 1 young. The temperature was then again lowered to 1.5-3.5 C. for 7 days resulting in the death of 8 old and no young. Therefore, in the entire experiment, 16 old (80 per cent) and 1 young (5 per cent) died, a highly significant difference ($P < 0.001$). That is, in this experiment, 20 per cent of the old and 95 per cent of the young survived a temperature of 1.5 to 3.5 C. for 7 days when taken to this temperature gradually. This is in marked contrast to Experiment 2, when no old or young mice survived beyond the fourth day when taken directly to a temperature of 2 to 4 C. Another interesting point lay in the difference in survival time at this low temperature. Whereas in Experiment 8 there were no deaths of either adapted young or adapted old mice *on the first day* of exposure to 1.5 - 3.5 C., 100 per cent of the unadapted old and 83 per cent of the unadapted young died *the first day* of exposure to 2 - 4 C. in Experiment 2.

TABLE 4. MEAN VALUE OF VARIOUS METABOLIC FACTORS AND BLOOD COUNTS OF YOUNG AND OLD FEMALE C57BL MICE DETERMINED AT 26 TO 27 C. PRIOR TO EXPOSURE TO COLD.

	Young		Old		Probability of Difference
Body Weight (Gm.)	24.9	(22)	29.7	(25)	$P < 0.001$
Food Intake (Gm./day)	3.5	(9)	3.9	(9)	$P > 0.20$
Oxygen Consumption (cc./hr/Gm.$^{3/4}$ at S.T.P.)	2.5	(9)	3.0	(9)	$P < 0.001$
Blood Sugar (mg.%)	93.0	(90)	80.1	(89)	$0.4 > P > 0.3$
Total Leucocyte Count $\times 10^3$ (per cu. mm. blood)	129	(30)	109	(29)	$0.10 > P > 0.05$
Erythrocyte Count $\times 10^4$ (per cu. mm. blood)	789	(30)	718	(29)	$0.05 > P > 0.02$
Eosinophil Count (per cu. mm. blood)	101	(30)	112	(29)	$0.6 > P > 0.5$
Lymphocytes Count (per cu. mm. blood)	12,179	(29)	8,632	(28)	$0.10 > P > 0.05$
"Polymorph" Count (per cu. mm. blood)	811	(29)	2,224	(29)	$P < 0.001$
Lymphocytes (% of Total Leucocyte Count)	93.1	(20)	80.0	(28)	$P < 0.001$
Polymorpho-leucocytes (% of Total Leucocyte Count)	6.4	(29)	18.9	(28)	$P < 0.001$

The number in the bracket gives the number of animals from which the adjacent mean was calculated.

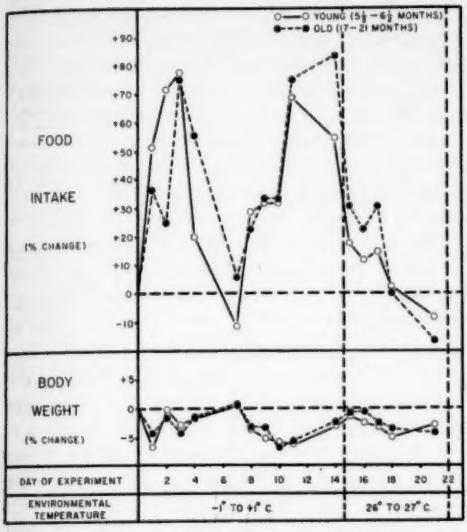

Fig. 1. Changes in body weight and food intake of young and old mice exposed to an environmental temperature of —1 to +1 C. The mice were housed together in groups of 15 or 16, and each point is the mean value obtained from at least 13 animals.

Body Weight and Food Intake: Pre-exposure values for the body weight and food intake are given in table 4

The results obtained from Experiment 1 in which the mice were housed together revealed no striking differences due to age in response to cold exposure. In both age groups the food intake increased for the first 3 days in the cold, then decreased for the next 4 days, and then increased again for the last 7 days (fig. 1). The body weight also did not show any striking change in either young or old in response to the cold (fig. 1). This is in agreement with the finding that there was no marked age difference in mortality of the animals in this experiment.

On the other hand, quite different results were obtained in Experiment 4 in which the mice were housed individually. Here, both groups of mice lost weight in the cold but the body weight loss was significantly greater in the old mice (fig. 2, $P < 0.001$). Moreover, on the first day after the mice were returned to the warm room, the body weight of the young reached and even exceeded the pre-exposure level, while that of the old mice did not reach the pre-exposure value even a week after their return to the warm room (fig. 2).

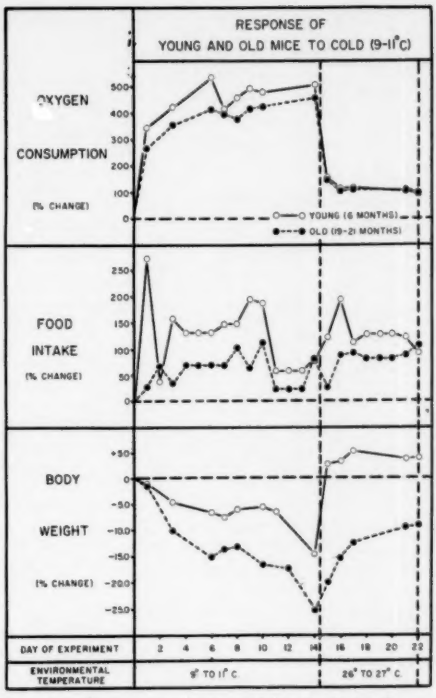

Fig. 2. Changes in body weight, food intake, and oxygen consumption of individually-housed young and old mice exposed to an environmental temperature of 9 to 11 C. Each point is the mean taken from 2 to 9 mice (from 6 animals on the average).

The food intake of both groups of mice in Experiment 4 increased on exposure to the cold, that of the young significantly more so than that of the old (fig. 2, $0.01 > P > 0.001$). During the week following the return of the animals to the warm room, no decline occurred in the food intake of either group to the pre-exposure level.

In Experiment 8 where the animals were *gradually* adapted to the cold the food intake of the mice increased above that of the previous warmer period (fig. 3). In general, the increase of the food intake was greater in the younger animals during the first 7 days of the experiment, and in this period, the difference was especially marked on the first day after the environmental temperature was lowered (fig. 3, days 1 and 4). A similar marked difference in food intake was observed in Experiment 4 when the mice were first exposed to the cold (fig. 2, day 1). However, when the temperature was further decreased after the mice of

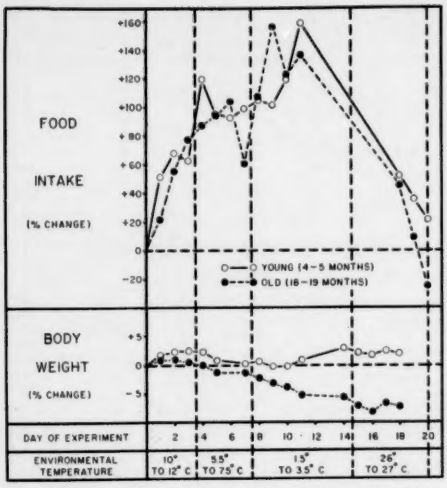

Fig. 3. Changes in body weight and food intake of individually-housed young and old mice exposed to a sudden drop in environmental temperature from 26 to 27 C. to 10 to 12 C. and then to a more gradual drop to 1.5 to 3.5 C. Each point is the mean obtained from 4 to 20 mice (from 17 animals on the average).

Experiment 8 had been in the cold room for 7 days, that is, after the mice had become adapted to some extent, the increase in food intake was at least as great in the old as in the young (fig. 3). Despite this, the body weight of the old decreased in this period, while that of the young did not.

Each time the temperature was lowered in this experiment, the young mice maintained their body weight at or slightly above the pre-exposure level throughout the stress period (fig. 3). However, the old mice maintained their body weight for only the first 5 days of the stress. Thereafter, their body weight showed a gradual decline and did not show any tendency to return to pre-stress values even after 4 days in the cold (fig. 3).

Oxygen Consumption: Oxygen consumption determinations were made on the mice of Experiment 4. The readings were taken after the mice had been fasted for 2 hours: a longer fast in the cold was avoided because it would probably have killed so many mice that the experiment would have been aborted. Control readings are shown in table 4. Exposure to cold increased the oxygen consumption in both young and old mice, but more so in the young (fig. 2, $P < 0.001$). This difference was apparent even on the first day of the stress when the oxygen consumption of both groups was already more than 200 per cent above the control values. Thereafter, the values continued to increase gradually to 400-500 per cent above normal at the end of the stress period, the young showing consistently higher values. Returning the mice to the warm room resulted in a marked drop of the readings, which, however, remained about 100 per cent above the pre-stress level during the week of the post-stress period. In this connection, the food intake also remained high in this period as described earlier (fig. 2).

Blood Sugar: Blood sugar determinations were carried out on the mice of Experiment 5 after a 2-hour fast. The control readings are shown in table 4. Exposure to cold resulted in a significantly greater increase in the blood sugar of the young animals as compared to the old (fig. 4, $0.02 > P > 0.01$). Returning the mice to the warm room resulted in a marked decline in the blood sugar to below the pre-exposure values in both groups. The post-stress values were the same in old and young. The values remained below normal even after the animals had been in the warm room for a week. This was in contrast to the findings in the oxygen consumption and food intake studies described earlier.

Blood Counts: Blood counts were taken in the mice of Experiment 6, and the control values are shown in table 4. The eosinophil count dropped to the same extent in both age groups when exposed to the cold, and in both groups the eosinophil count rose towards the end of the stress even before the animals were

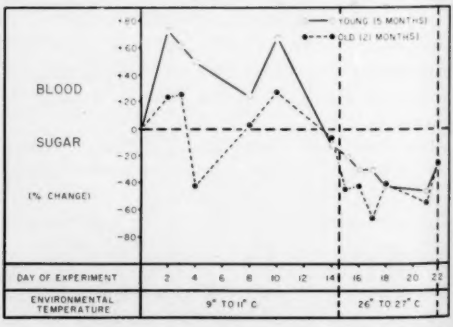

Fig. 4. Changes in the blood sugar of individually-housed young and old mice exposed to an environmental temperature of 9 to 11 C. Each point is the mean obtained from 2 to 5 animals (from 4 animals on the average).

returned to the warm room (fig. 5, $P > 0.20$). In the warm room the counts showed some wide variations above and below the pre-stress levels. Sharp peaks have been shown to occur after cessation of adrenocortical stimulation in certain other physiologic functions known to be influenced by the adrenocorticoids (8, 15).

The total leucocyte count also decreased on exposure to cold, but significantly more so in the young than in the old (fig. 5, $0.01 > P > 0.001$). The lymphocyte count parallelled the total leucocyte change, and here also the decline in the cold was significantly greater in the young (fig. 5, $0.05 > P > 0.01$). Moreover, the number of polymorpho-leucocytes of the young increased while that of the old decreased in the cold (fig. 6). Wide variations, similar to that seen in the eosinophil count, were observed in the post-stress period. However, the counts of the young remained above that of the old (fig. 6). Finally, during the stress

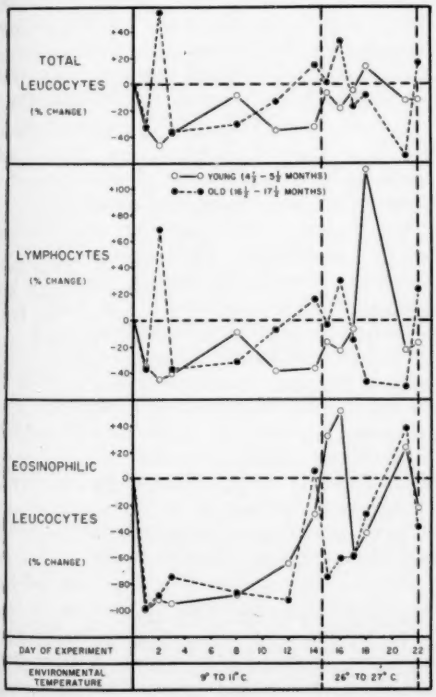

Fig. 5. Changes in total leucocyte, circulating lymphocyte, and eosinophil cell counts of individually-housed young and old mice exposed to an environmental temperature of 9 to 11 C. Each point is the mean value obtained from 1 to 5 mice (from 3 animals on the average).

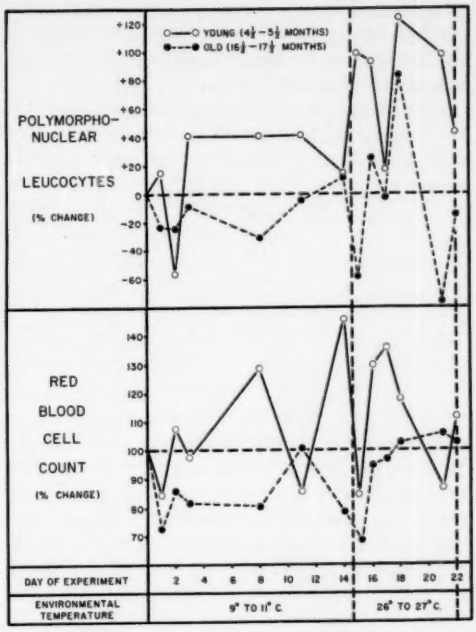
YOUNG (4½ – 5½ MONTHS)
OLD (16½ – 17½ MONTHS)
POLYMORPHO-NUCLEAR LEUCOCYTES (% CHANGE)
RED BLOOD CELL COUNT (% CHANGE)
DAY OF EXPERIMENT
ENVIRONMENTAL TEMPERATURE
9° TO 11° C.
26° TO 27° C.

Fig. 6. Changes in polymorphonuclear leucocyte and erythrocyte cell counts of young and old mice exposed to an environmental temperature of 9 to 11 C. Each point is the mean value obtained from 1 to 5 mice (from 3 animals on the average).

period the red blood cell count declined in the old, but increased in the young, the difference due to age being significant (fig. 6, $P < 0.001$). During the week in the warm room after the exposure to cold the erythrocyte values gradually returned to normal in the old mice while that of the young continued to fluctuate.

Autopsy Findings: The following are some of the more consistently observed findings among the mice which died during the cold exposure: (a) little or no food in the stomach; this was observed more frequently in the old than in the young; and (b) apparently hyperemic brains. Pneumonia was observed in about 5 per cent of the mice exposed to cold. These anatomic findings are provisional and will be investigated more thoroughly in a subsequent study.

DISCUSSION

The most significant finding of the experiments reported here was that the mortality rate of old mice upon exposure to cold was significantly greater than that of young. To demon-

strate this difference, two conditions had to be fulfilled: first, the cold temperature to which the animals were exposed had to be carefully selected. In our experiments the optimal temperature range for this purpose was 6 to 7 C., but highly significant differences due to age were also observed at 9 to 11 C. However, when unadapted mice were exposed to 2 to 4 C. or to 15 to 17 C., such age differences in mortality did not occur. Second, the mice had to be housed individually. When housed together in groups of 15 or 16, no age difference was apparent even at a temperature of -1 to $+1$ C. This is undoubtedly due in large part to the physical warmth which the animals imparted to each other as they huddled together.

In analogy with the procedure followed in pharmacologic studies, an attempt was made in figure 7 to estimate the temperatures which will kill 50 per cent of young and of old mice under the experimental conditions of our laboratory. This temperature was estimated as being between 4 and 5 C. for the young and between 9 and 10 C. for the old.

That old mice can adapt to the cold is apparent on comparison of the result of Experiment 2 with that of Experiment 8. In the former, 100 per cent of old mice died on the first day of exposure to 2 to 4 C. in highly significant contrast to the 0 per cent deaths of old mice on the first day when taken to this temperature more gradually as in Experiment 8. Furthermore, of the 12 *cold-adapted old* mice exposed to 1.5 to 3.5 C. for 7 days, 4 were still alive at the end of the stress period, while all of the 19 *cold-adapted young* mice were still alive after 7 days under the same conditions.

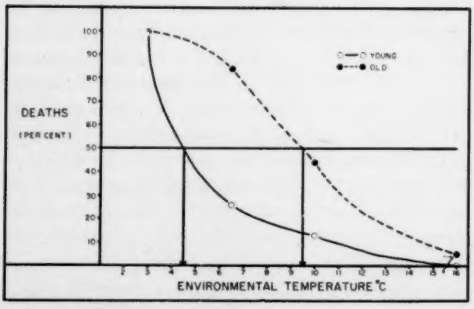

Fig. 7. The effect of a two-week exposure at various low temperatures on the mortality rate of different groups of individually-housed young and old mice. Each point is the mean of at least 20 animals.

Therefore, adaptation to cold is less effective in the old.

From considerations of size alone, the old would have been expected to have fared better in the cold than the young, as the former were decidedly larger (table 4). That is, the ratio of surface area to volume was less in the old then in the young and this by itself should have operated to the advantage of the old when exposed to the cold. That despite this the old died more readily in the cold points to the over-riding effect of other factors, namely, the lesser efficiency of the homeostatic mechanisms in the old. The exact nature of this defect or defects is presently unknown and is one of the ultimate objectives of this reasearch. As a beginning in this direction, a variety of biologic functions were studied at the specifically chosen temperature of 9 to 11 C. A lower temperature resulted in too high a mortality rate in both age groups, leaving too few animals for study, whereas at a higher temperature the stress might not have been sufficient to reveal age differences in the biologic functions studied. This was inferred from the fact that there were very few deaths and no significant differences due to age in mortality rate on exposure to 15 to 17 C.

As a result of exposure to cold, the oxygen consumption, food intake, and blood sugar increased in both age groups, but significantly more in young than in old; the body weight decreased in both groups but significantly less in the young. The increased food intake was not sufficient to prevent body weight loss in either group. This deficit was much more apparent in the old. In other words, catabolic as well as anabolic mechanisms increased in both age groups under stress, the former more than the latter, resulting in a metabolic deficit. However, this deficit was significantly greater in the old than in the young. In view of the nitrogen loss into the urine which regularly accompanies stress (31), a greater negative nitrogen balance in the old than in the young would be expected under the conditions of our experiment.

The total leucocyte count and the circulating lymphocyte and eosinophil counts decreased in both age groups during exposure to cold, while the number of polymorphonuclear cells increased. In the mouse, in contrast to man, the leucocytes consist mostly (80 to 90 per cent) of lymphocytes (table 4). Therefore, the decrease in total leucocyte count was due to the

decrease in the lymphocyte count even though the polymorphonuclear count increased. The eosinopenia and lymphopenia are well-known responses to stress and are due to the increased secretion of 11-oxygenated adrenocorticoids under stressful conditions (12). However, a more complex situation seems to exist in the case of the neutrophilia, which can be produced by adrenal cortical extract (19), but which also is obtained with ACTH in adrenalectomized animals (10).

As for differences due to age, the percentage decreases in the circulating lymphocytes and consequently in the total leucocytes was significantly greater in the young. This may indicate a greater secretion of corticoids of the adrenal cortex in the young as compared with the old, or may be due to a greater resistance of the lymphocytes of the old mice to destruction both in the blood and lymphoid tissues (11), and/or a greater resistance to peripheral removal of lymphocytes (13).

On the other hand, there was no significant difference in the percentage decrease of the circulating eosinophil count between the two age groups on exposure to cold. By itself, this would seem to suggest that there may be no difference due to age in the corticoid output under our experimental conditions. However, the degree of eosinopenia on exposure to cold was maximal in both groups, particularly in the early days of the stress. In this connection it is of interest to note that in the Speirs-Meyer eosinopenic bio-assay for adrenal corticoids, there is no significant difference in eosinopenic response to 24 and 96 micrograms of cortisone (33), although the log dose—response curve at lower dosages is a straight line of steep slope. Hence, the possibility remains that in our experiments there actually was a significant difference in corticoid output between the old and young mice in the cold, but that the amounts of corticoids secreted in both groups were too great for any significant differences to become apparent by eosinopenic assay.

The erythrocyte count of the young showed an over-all increase in the cold, and this could be due to hemoconcentration known to occur in the cold (26). On the other hand, the red blood count of old mice decreased in the cold and this suggests that hemoconcentration did not occur in them or was obscured by a less active erythropoiesis or a more active erythrolysis.

If hemoconcentration did occur in the young, it would also account in part at least for the increase of the polymorphonuclear cells in the cold. It could also mean that the number of circulating lymphocytes and eosinophils of the young in the cold was less than actually counted, making differences due to age still greater.

When the surviving animals were returned to the warm room, a reversal of several of the physiologic variables was observed. Thus, in both groups the oxygen consumption and the blood sugar decreased, while the body weight increased. Hence, in contrast to the stress period when catabolism predominated in both groups, resulting in body weight loss, now anabolism predominated, resulting in body weight gain. This was achieved in part at least by a high level of food intake, which remained at the level reached in the cold. This increased appetite in the post-stress period may have been due to the low blood sugar. Furthermore, the predominance of anabolism must have been greater in the young than in the old as the body weight gain was greater in the former. As expected from this the food intake was also higher in the young than in the old. This also suggests that nitrogen retention under these circumstances was greater in the young than in the old.

All the blood counts showed considerable fluctuation in both age groups when the animals were returned to the warm room. Post-stress peaks have also been reported for other functions known to be under adrenal cortical control, for example, salivary sodium concentration (15) and the concentration of sodium and chloride ions in sweat (8). As the red blood cell count also showed considerable variation in this period in the young mice, changes in blood volume may have been another factor in the post-stress fluctuations of the blood counts in this group. However, the phenomena described here were seen only during the first week after the mice were returned to the warm room. A more prolonged observation period may have resulted in further changes not reported here.

Of the biologic functions studied several are commonly thought of as being under the control of the adrenal glands. Thus, the decrease in eosinophils and lymphocytes, the increase in polymorphonuclear leucocytes, and the decrease in body weight can be produced by corti-

sone-like compounds. The increase in the oxygen consumption can be ascribed to the increased secretion of adrenaline from the adrenal medulla early in the cold and by thyroid hormones later on. The blood sugar increase can be produced by an increased output of adrenal medulloids as well as corticoids.

However, most of these changes have also been produced by stimulation of autonomic centers in the hypothalamus. Thus, there are areas in the hypothalamus, the stimulation of which leads to eosinopenia (1), lymphopenia (17) and polymorphonuclear leucocytosis (27). Hyperglycemia has also been produced by hypothalamic stimulation (2), and this, as well as the hypothalamic-induced eosinopenia and lymphopenia, may have been mediated via the pituitary and adrenal cortex. However, the increased food intake cannot be ascribed to increased activity of the adrenal glands, but has been shown in numerous studies to be under hypothalamic control (5). Also, as indicated above, certain of the endocrines play a role in the increased oxygen consumption which occurs on cooling, but the main reason for this increase in unacclimatized animals is the increased skeletal muscular activity (30) and this is under nervous control (20). Finally the hemoconcentration which takes place on exposure to cold (26) which was suspected to be responsible for the polycythemia and perhaps also for the neutrophilia of young mice in the cold, is also definitely under nervous control (3, 26).

The changes in the functions described in this report as well as many other functions not mentioned here undoubtedly occur in an integrated manner in the animal organism exposed to stress. The nature of the integrative mechanism is actively being investigated and discussed at present (7, 19, 24), and although there is some disagreement as to details, it is generally believed that stress leads first to the stimulation of center(s) in the hypothalamus via humoral or neural pathways. Thereupon, impulses travel down the sympathetic nervous system, stimulating the adrenal medulla to release epinephrine and/or arterenol, which besides having peripheral effects, also stimulates the posterior pituitary, which then stimulates the anterior pituitary to increase its output of ACTH (29). In this way the adrenal cortex is stimulated to increased secretion of corticoids, which then act on target tissues, exerting its anti-stress effects, some of which were described in this paper. However, it must be emphasized that there are aspects of the integrative actions of the nervous system in the adaptation to cold which are not mediated via the endocrines.

Whatever the mechanism(s) of the integration of these functions, our experiments clearly demonstrated that this was not achieved as efficiently in the old as in the young, as revealed by the failure of the old animals to survive conditions not fatal to young. Moreover, our experiments also demonstrated that the fatal failure of old mice to adapt to cold generally occurred early in the stress period, that is, in the alarm phase of the Selye's adaptation syndrome (32). Old animals which survived the first two days of cold exposure had a good chance of surviving the remaining 12 days of stress. In this connection it should be pointed out that the biologic functions investigated were generally conducted on a selected group of old mice, that is, on those mice which survived the alarm phase. Hence, the differences in the various biologic functions would probably have been even greater if measurements of old mice which died could have been included. However, the fact that even this select group of old mice did not respond to the stress as favorably as the young suggests that in this select group too there was present some homeostatic defect which, however, did not have fatal consequences, under the conditions of our experiments.

SUMMARY

1. Old C57BL female mice showed a very significantly higher mortality rate on exposure to cold than mature young females. This age difference was made apparent in our experiments only when individually housed animals were exposed to a certain range of low temperatures. The optimal temperature for this purpose was 6 to 7 C., although very highly significant differences were also observed at 9 to 11 C.

2. Temperatures which killed 100 per cent of unadapted old mice within 24 hours, killed none of the old mice within this time limit when they were taken to this temperature gradually, an indication that old mice can adapt to the cold. Two-thirds of adapted old mice died after a week at a temperature which failed to kill any adapted young mice. Therefore, adaptation to cold is less effective in the old.

3. Exposure to a "cold room" temperature of 9 to 11 C. resulted in significantly greater increases in the oxygen consumption, food intake, and blood sugar in the young than in the old; body weight decreased significantly less in the young. Blood lymphocyte counts decreased reliably more in the young than in the old and the erythrocyte count increased significantly more in the young. There was no significant difference due to age in the eosinopenic or neutrophilic response to cold.

4. Theoretic implications of these findings are discussed.

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CHRONIC SODIUM CHLORIDE TOXICITY IN THE ALBINO RAT. III. MATURITY CHARACTERISTICS, SURVIVORSHIP, AND ORGAN WEIGHTS.*

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The induction of hypertension by increased ingestion of sodium chloride has been demonstrated in experimental animals under conditions of restricted fluid intake (6, 14) and with drinking water provided ad libitum (5, 12). In an attempt to define the subtle and long term effects of various levels of intake of sodium chloride, we have maintained rats from youth throughout life on diets containing several constant levels of NaCl. This report, abstracts of which have appeared elsewhere (13, 17, 18), describes the maturity characteristics, longevity, and terminal data of this sample.

MATERIALS AND METHODS

The experimental regimen has been described (11). Two hundred and fifty-one male, albino rats of the Sprague-Dawley strain were placed on purified rations and demineralized water ad libitum. The rats were 5 weeks of age and weighed an average of 119 Gm. The rations contained 9 different levels of sodium chloride, expressed as per cent of the diet, as follows: ration I (low Na diet) about 0.01; ration II (control diet), 0.15; ration III, 2.8; ration IV, 5.6; ration V, 7.0; ration VI, 8.4; ration VII, 9.8; ration VIII, 14.0; and ration IX, 21.0. Each dietary group embraced 30 rats, except Group I, which contained 11, and Groups V and VII, comprised of 29 and 31, respectively. Measurements of systolic blood pressure were made by the cuff method of Kersten and associates (10). Electrocardiograms were taken with a Sanborn four-channel direct writing recorder at a paper speed of 50 mm. per second using subcutaneous needle electrodes. The rats were permitted to die or were sacrificed in extremis. Autopsies were performed as soon after death as feasible.

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RESULTS

Maximum Body Weight: The maximum body weight attained was related to the amount of salt in the diet. Fig. 1 shows that the maximum body weight decreased at the lowest NaCl intake as well as at all dietary levels above that of the control group (0.15 per cent). The mean of the individual maximum body weights for the control group was significantly larger than the mean for the low-Na group ($P=0.015$) as well as for any of the other groups ($P < 0.01$). Obesity, most pronounced in the control group at the intake level of 0.15 per cent NaCl, was observed in animals at all levels up to and including 5.6 per cent NaCl. It was not seen among rats eating 7.0 per cent or more of NaCl in the diet. Two exceptionally obese control rats achieved maximum weights of 1008 Gm. and 974 Gm.

Time of Attainment of Maximum Body Weight: The several groups differed with respect to the length of time required to attain maximum body weight (fig. 1). The control rats continued to grow in weight for an average of 17.4 months of the experimental regimen. This value was not significantly greater than the average for the low-Na group (14.4 months) or for the 2.8 per cent NaCl (15.1 months) and 5.6 per cent NaCl (16.4 months) groups. However, the control value did exceed significantly the means for each group consuming 7.0 per cent or more of NaCl.

Systolic Blood Pressure at Maximum Body Weight: The mean systolic blood pressure measured during the month in which the maximum

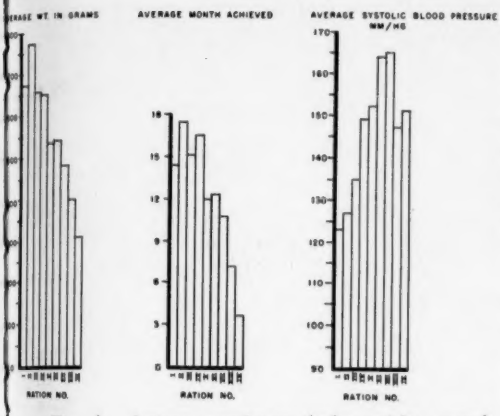

Fig. 1. Average maximum body weight, month achieved, concurrent systolic blood pressure: by rations.

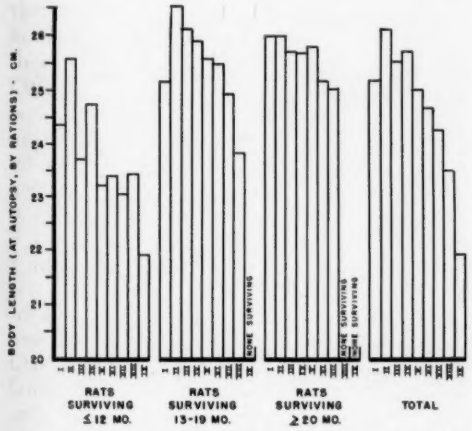

Fig. 2. Average body length (cm.) at autopsy: by rations.

body weight was attained is shown in figure 1. The differences between the mean of the control group and of the low-Na group is not significant ($P = 0.15$). The differences between the mean of the control group and of each of the other groups are highly significant statistically ($P < 0.01$). The reader should recognize that the blood pressure at maximum body weight is not synonymous with *maximum* blood pressure exhibited during the life-span. This distinction is important in appraising the highest levels of salt feeding in groups in which the maximum blood pressures were well above the levels illustrated in figure. 1.

Body Length: The mean data for body length at death are given in figure 2. The measurement was made from the tip of the nose to the tail base at the time of autopsy. For the entire sample the control rats were significantly longer than rats raised on the low-Na diet ($P = 0.03$). The rats on the first two elevated levels of salt (2.8 per cent and 5.6 per cent NaCl) did not differ significantly in length from the controls. However, all groups eating 7.0 per cent or more of NaCl were significantly shorter than were the control animals ($P < 0.01$). When the gross sample was subdivided on the basis of time of survival, a similar pattern of body lengths prevails for the groups on levels of 7.0 per cent or more salt intake. The numbers of animals in many of the subgroups are too small for meaningful significance tests, but the trends are unmistakable. The data in figure 2 suggest that the few animals on 0.01 per cent NaCl which survived more than 20 months did attain a length equal to that of the controls. The small number of rats in this dietary group which survived to more than 20 months makes the finding insecure until more animals can be observed.

Over-all evaluation of the data leads to the conclusion that rats consuming 0.15 per cent NaCl (the control group) grew to a greater size —both greater body weight and length—than did rats eating either 0.01 per cent NaCl or 2.8 per cent or more NaCl. Furthermore, the period of growth was longest for the control group. Systolic blood pressure at the time of attaining maximum weight was lowest in Groups I and II, that is, at levels of 0.01 and 0.15 per cent NaCl, respectively. It was significantly higher in all other groups (fig. 3).

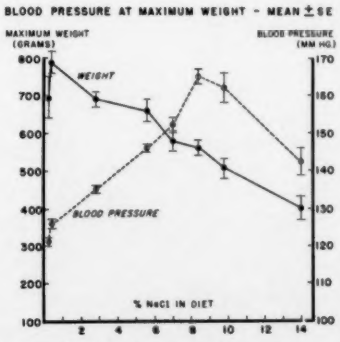

Fig. 3. Systolic blood pressure at maximum weight.

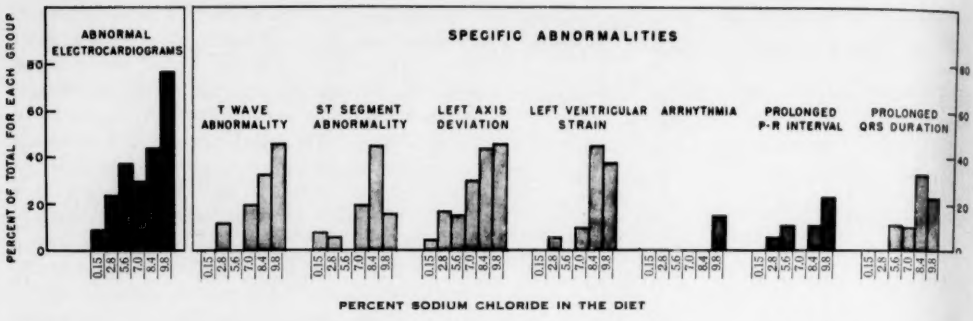

Fig. 4. Percentage incidence of abnormal electrocardiograms in rats eating various levels of sodium chloride for 19 months.

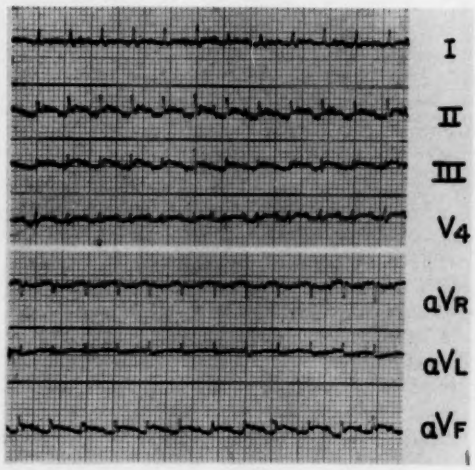

Fig. 5. Normal control, diet 0.15 % NaCl, systolic blood pressure—122, heart rate—460/min.

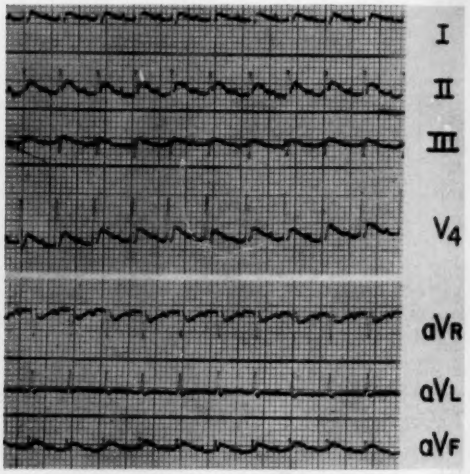

Fig. 6. Mild hypertension, diet—5.6 % NaCl, systolic blood pressure—144, heart rate—434/min.

Electrocardiograms: Electrocardiographic tracings were made on all rats surviving 19 months of the experimental regimen. Blind analysis of the 90 electrocardiograms revealed in all groups an incidence of abnormalities which paralleled the degree of sustained arterial hypertension and the concentration of NaCl intake (fig. 4). Observations were limited to diets containing not more than 9.8 per cent NaCl, since rats eating 21.0 per cent NaCl were all dead at 9 months, and those eating 14.0 per cent NaCl at 15 months. It is noteworthy, however, that about 80 per cent of the rats eating 9.8 per cent NaCl exhibited abnormalities. Selected tracings show animals with normal systolic blood pressure (fig. 5), mild hypertension (fig. 6), moderate hypertension (fig. 7), and severe hypertention (fig. 8). Clearly the incidence of cardiac damage which occurred in these "elderly" rats was related to the amount of NaCl ingested in their diet.

Longevity: Data on per cent survival of the controls (0.15 per cent NaCl) and of the 7 groups eating higher NaCl levels, 2.8 to 21.0 per cent, are presented in fig. 9. Levels of 21.0 and 14.0 per cent NaCl resulted in high mortality early in the course of the experiment. The levels from 7.0 to 9.8 per cent NaCl re-

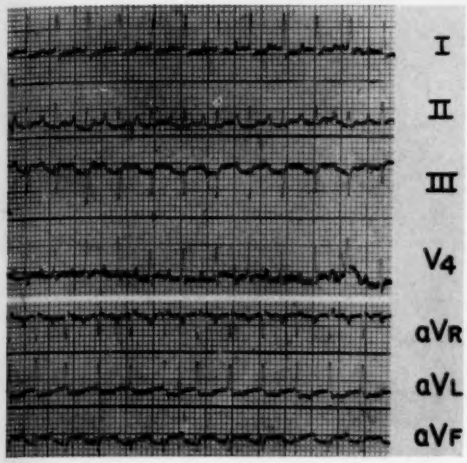

Fig. 7. Moderate hypertension diet—8.4 % NaCl, systolic blood pressure—176, heart rate—496/min.

sulted in a decreased survival, detectable from about the fifth month of the experiment. Nevertheless, some 65 per cent survival persisted in this group throughout the first year of the study.

The other levels (0.15, 2.8 and 5.6 per cent) present the especially striking feature of identical survival until the fifteenth or sixteenth month of exposure to the diet—that is, until the period of life corresponding to late maturity or early senescence. From this point onward the survival of these rats eating 2.8 or 5.6 per cent NaCl was definitely less favorable than that of rats consuming the control level of 0.15 per cent NaCl. Although the data on the survival of the group on 0.01 per cent NaCl are limited, they show a reduction in survival and longevity beginning in the 16th experimental month (fig. 10).

Organs Weights: Analysis of data on wet organ weights at death must take into account the influences of the variation in body weight and the variation in age of the animals at death. In order to do so, the data have been grouped on the basis of length of time the animals were subjected to the experimental regimen prior to death ($\leqq$ 12 mo.; 13-19 mo.; $\geqq$ 20 mo.), and the organ weights are expressed as weight of the organ in grams per kilogram of body weight. The data on heart, kidney, and adrenal glands are so presented.

a. *Heart:* In the total of the groups of animals the mean cardiac weight increased with the increase in quantity of NaCl in the diet, the greatest cardiac hypertrophy being evident in the dietary groups VII-IX (fig. 11). These increments are consistent with the data on hypertension in the total groups. Cardiac hypertrophy, which parallels the dietary increments of NaCl, was clearly evident in those rats surviving late ($\geqq$ 20 mo.) in the experiment, but was less marked at intermediate levels of intake in those animals dying or sacrificed during the first year. In the groups dying in this early period there is no apparent difference in relative heart weight until the NaCl intake is at 7.0 per cent (Ration V). In the survivors to 20 months and beyond, the increased relative cardiac weight is apparent at 2.8 per cent NaCl in the diet (Ration III). Data on absolute cardiac weight were similar in pattern, although

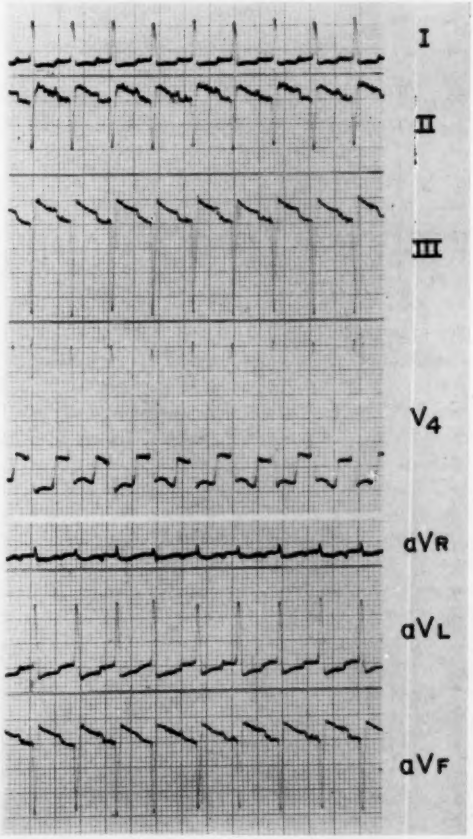

Fig. 8. Severe hypertension, diet 8.4 % NaCl, systolic blood pressure—191, heart rate—378/min.

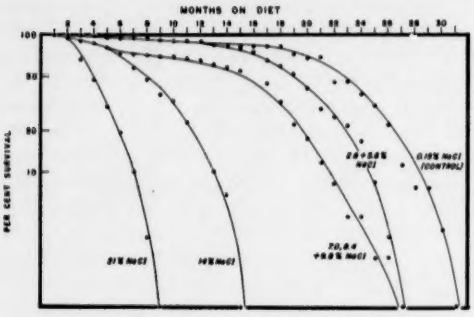

Fig. 9. Effect of increased NaCl on survival.

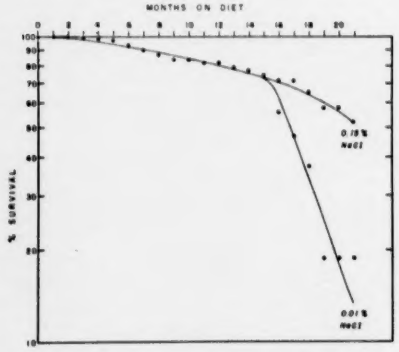

Fig. 10. Effect of low dietary NaCl on survival.

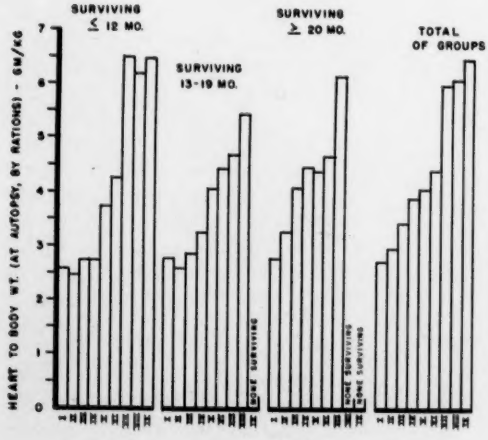
SURVIVING ≤ 12 MO.
SURVIVING 13-19 MO.
SURVIVING ≥ 20 MO.
TOTAL OF GROUPS
HEART TO BODY WT. (AT AUTOPSY, BY RATIONS) - GM/KG
NONE SURVIVING

Fig. 11. Heart weight (Gm./Kg.) by rations.

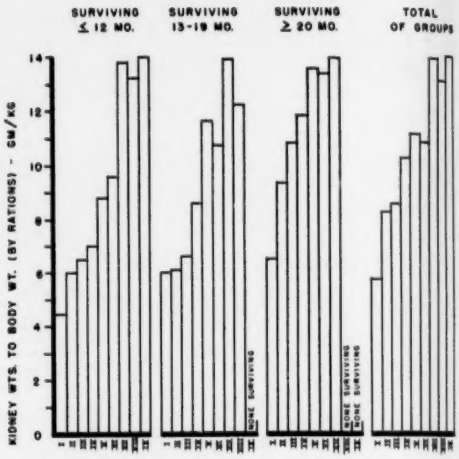

Fig. 12. Kidney weights (Gm./Kg.) by rations.

less striking in degree for Rations I-VI (0.01-8.4 per cent NaCl). At the highest levels of NaCl (Ration VII-IX) the absolute cardiac weights were abnormally high, but the increased weight did not continue to parallel the increment of NaCl in the diet.

b. *Renal Weight:* The pattern of renal hypertrophy (fig. 12) differed but slightly from the pattern of cardiac hypertrophy.

c. *Adrenal Weights:* The relative adrenal weight changes (fig. 13) showed a less consistent pattern than those of heart and kidney. Rats at the lowest salt intake level (0.01 per cent) had enlarged adrenals if they survived less than 20 mo. The smallest relative adrenal weights were found most consistently in the control (0.15 per cent NaCl) and Ration III (2.8 per cent NaCl) animals. The greatest increase in relative adrenal weight usually occurred in the rats at the high salt intake levels (7.0 per cent to 21.0 per cent NaCl) regardless of the length of time the rats had been on the experiment. That these relative weights are greatly influenced by the body weights is evident from inspection of fig. 14 which shows the actual average weight of the adrenals in milligrams at autopsy for the various survivors' age and diet groups. Here one observes, in animals dying during the first year of experiment, an increased adrenal size related to the amount of NaCl in the diet, but a converse relationship in those dying in the later period ($\geqq$ 20 months) of the experiment.

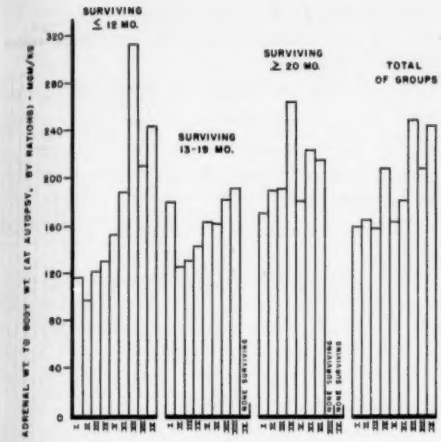

Fig. 13. Adrenal weights (MGM/Kg.) by rations.

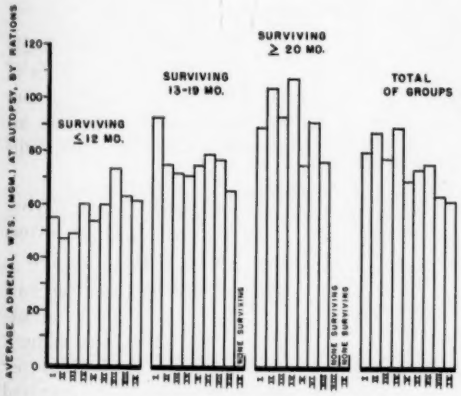

Fig. 14. Absolute adrenal weights by rations.

DISCUSSION

The possibility of inducing a harmful excess of salt into the body by feeding salt generally has been discounted, at least in normal animals. It has been known for several years, however, that a high-salt intake potentiates the hypertension induced by desoxycorticosterone as pointed out by Selye and associates (15). Conversely, as shown by Allen and Sherrill (1), Kempner (9), and many other clinicians, it has been demonstrated beyond cavil that a low-salt diet can substantially reduce the blood pressure in many hypertensive patients. Therefore, the possibility exists that long-continued excessive salt intake may be deleterious even though it does not lead to obvious edema and plethora.

The data presented here demonstrate that in the male albino rat diets both high and low in sodium chloride content have unfavorable physiologic and nutritional effects. The preponderance of the data deals with rats chronically fed high levels of NaCl. Untoward effects were encountered using the following criteria: (a) maximum body weight; (b) body length; (c) growth period, i. e., the time required to achieve maximum body weight; (d) systolic blood pressure at the time of maximum body weight; (e) the incidence of electrocardiographic abnormalities; and (f) longevity. The small sample studied on the low-Na diet reduces the reliability of the data at the opposite extreme. However, the rats on the low-Na diet fared less well than the control animals when judged by three criteria: (a) maximum body weight; (b) body length; and (c) longevity. Since the rats on Ration I (0.01 per cent NaCl) were housed in the same room with the rats feeding on high levels of NaCl, it is possible that the low-Na group obtained additional traces of salt adventitiously, e. g., from dust in the air, and hence experienced a more favorable survival and growth than would have been observed under more severe restrictions.

The electrocardiograms were analyzed and the abnormalities tabulated completely independently. The observer had no knowledge whatsoever of the diet or blood pressure of any of the animals. The 90 electrocardiograms analyzed included a large number of completely normal tracings of which figure 5 is representative. The abnormal tracings on the other hand exhibited an array of changes not too dissimilar from those which might appear in a series of electrocardiograms taken on human hypertensive patients. The significance of the ST and T wave changes and of simple left axis deviation is difficult to assess. The left ventricular "strain" pattern, however, is so similar to many tracings in human hypertensives that true cardiac abnormality can be assumed. The fact that these electrocardiographic abnormalities correlated well with the heights of arterial blood pressure and with the size of the hearts at autopsy provides further confirmation of the validity of these measurements.

The data on heart weight appear to be consistent with that of Stein and Barnes (16) who reported that among 111 cases once normotensive and later hypertensive, cardiac hypertrophy seemed to be related directly to the sever-

ity of the hypertension. Berg and Harmison (2) failed to detect significant cardiac hypertrophy in a sample of elderly male rats which developed hypertension spontaneously. The hypertension in every case was related to renal disease, and it seems likely that a different mechanism was involved than in the rats in which hypertension was induced through feeding increased levels of NaCl.

Kaunitz (8) reported that diets high in NaCl or Cl caused marked renal hypertrophy in young rats. In the same animals adrenal hypertrophy was present although to a less striking degree. These data appear to be consistent with our observations in elderly rats. It may be that the elderly rats experienced some adrenal hypertrophy in all groups due to non-specific causes associated with death.

The optimum level of NaCl in the diet of the rat remains to be defined. The 0.15 per cent level used in Ration II (control) was taken from the Hubbell, Mendel, and Wakeman (7) formula. Although the control rats performed better than any of the experimental groups using a number of criteria, it is probable that this level is not ideal. Possibly the optimum level lies between Rations II and III (0.15 per cent and 2.8 per cent NaCl).

In comparison with these animal data it is interesting to note the work of Dahl and Love (3) who observed a positive correlation between the incidence of hypertension and the salt-adding habits of 547 human adults. The average intake of NaCl for a normal adult is estimated at 7 to 15 Gm. per day (4). Based on a 2500 Cal. diet containing 500 Gm. of dry matter, the typical human diet may contain from 1.4 to 3.0 per cent of NaCl. In other words, a fraction of the populace consumes dietary salt at a level greater than was included in ration II. In the present studies intakes of NaCl in excess of this level resulted in retarded growth, elevated systolic blood pressure, higher incidence of electrocardiographic abnormalities, relative cardiac hypertrophy, and reduced life span of the rat.

SUMMARY

Male rats were fed purified diets containing 9 different levels of NaCl (0.01 to 21.0 per cent of diet) and water ad libitum from 5 weeks of age throughout life. The following conclusions are drawn:

Control rats grew larger (weight and length) than rats consuming diets high or low in NaCl content.

Control rats continued to gain weight for longer periods of time than rats consuming diets containing 7 per cent or more of NaCl.

The systolic blood pressure measured at the time of maximum body weight achievement was related directly to the NaCl content of the diet.

Abnormal electrocardiograms correlated well both with the per cent of NaCl in the diet and with the height of systolic blood pressure. The electrocardiographic abnormalities were remarkably similar to those encountered in human hypertensive patients.

The relative weights of heart, kidney, and adrenal glands were correlated with the NaCl content of the diet.

Control rats survived longer than rats consuming diets high or low in NaCl content.

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AGING AND THE LABILITY OF IRREPLACEABLE MOLECULES*

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Aging is usually treated as a biologic rather than a chemical process. This viewpoint is well expressed in the statement by Lansing (27) that "The old organism does not contain old colloids; it contains newly formed colloids of an old character." This paper will explore the alternative possibility, namely that the aging of an organism is primarily caused by molecular changes in nucleic acid and protein.

An evaluation of the aging problem in these terms seems worth while because aging, like radiation injury, must ultimately have a biochemical explanation. We will treat the possible role of the molecular aging of DNA and collagen in respect to the life span of an animal, and the molecular aging of hemoglobin in relation to the life span of the red blood cell.

The nucleic acid and protein of the body are subject to a variety of insults—chemical, mechanical, and thermal. Radiation is now recognized as an additional hazard. Of all agents capable of altering macromolecules, the most ubiquitous and probably the most important is heat. The thermal energy of the atoms that form the molecular structure of living organisms continuously vibrates these atoms back and forth, twisting and stretching chemical bonds, and exerting a steady influence for the alteration of structure.

THE THEORY OF ABSOLUTE REACTION RATES

A mathematical treatment of the relationship between temperature and reaction rates applicable to the denaturation of protein and DNA was first developed by Arrhenius (2) and later refined by Glasstone, Laidler, and Eyring (16). The application of this theory to biologic problems is discussed in some detail by Johnson, Eyring, and Polissar (23).

According to the theory of absolute reaction rates, the inactivation or denaturation of a protein or macromolecule requires a preliminary activating step which involves an appreciable increase in heat content, $\triangle H$, and entropy, $\triangle S$. Once the molecule enters the activated state, inactivation and denaturation may proceed spontaneously. The rate-limiting reaction is considered to be the initial activation.

The fundamental equation of this theory of absolute reaction rates describes how K, the constant describing the velocity of a reaction, varies with temperature. This equation is

$$K = \frac{kT}{h} \kappa K', \tag{1}$$

k is Boltzman's constant, h Planck's constant, T the absolute temperature, K′ the equilibrium constant of the reaction leading to the formation of an activated complex and κ a transmission coefficient expressing the probability that the activated complex will form the products of the reaction in question.

In practice, it is necessary to make a number of transformations. κ the transmission coefficient, is usually assumed to equal unity. And since

$$\Delta F = -RT \ln K' \tag{2}$$

and

$$\Delta F = \Delta H - T\Delta S \tag{3}$$

the relationships

$$K = \frac{kT}{h} e^{-\Delta F/RT}, \tag{4}$$

and

$$K = \frac{kT}{h} e^{-(\Delta H/RT - \Delta S/R)} \tag{5}$$

may be derived.

Inactivation usually proceeds as a unimolecular reaction, since a plot of the logarithm of the remaining activity against time gives a straight line, the slope of which is the reaction velocity constant K. This reaction velocity contant varies as a function of temperature.

From Equation No. 4 it follows that

$$\Delta F = -RT \ln\left(\frac{K}{\frac{kT}{h}}\right) \tag{6}$$

From Equation Nos. 3 and 6 it is possible to determine $\triangle H$ and $\triangle S$ by plotting $\triangle F$ against T or by solving simultaneous equations. $\triangle H$ and $\triangle S$ are assumed to be constants applicable over a rather wide range of temperatures. The validity of this assumption is dis-

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cussed by Johnson, Eyring, and Polissar (24).

It is often convenient to treat reaction rates in terms of t ½ or the time required for a reaction to be 50 per cent complete. The relation between t ½ and K for first order reactions is

$$K = \frac{0.6932}{t_{\frac{1}{2}}} \quad (7)$$

From Equation No. 5 it follows that

$$\frac{0.6932h}{kTt_{\frac{1}{2}}} = e^{-(\Delta H/RT - \Delta S/R)} \quad (8)$$

which can be used to calculate △ H and △ S since

$$\ln \frac{0.6932h}{kTt_{\frac{1}{2}}} = -\left(\frac{\Delta H}{RT} - \frac{\Delta S}{R}\right) \quad (9)$$

is of the form y = mx + b where the slope of the line obtained by plotting the logarithm on the left against 1/T has a slope equal to △ H/R.

COLLAGEN

In discussing molecular aging, it is pertinent to inquire whether molecules of collagen might undergo some chemical change with senescence which would alter the physiologic properties of connective tissue. This question is of particular interest in the case of human beings whose life expectancy is gradually increasing, and who are afflicted with a variety of degenerative diseases of collagenous tissue in their later years. For the molecular aging of collagen to be considered significant, it would be necessary to establish three points: (a) that the changes which occur in collagen with time would have an adverse physiologic effect, (b) that these changes occur under physiologic conditions at a significant rate, and (c) that the rate of collagen replacement is sufficiently slow to allow altered molecules to accumulate. In regard to postulate (a) it may be stated that a gradual thermal denaturation of collagen would definitely alter its chemical and physical characteristics, and this in all probability would leave an adverse physiologic effect. In regard to postulate (b), it is difficult to establish experimentally the rate at which collagen denaturation might proceed under physiologic conditions. In vitro measurements of collagen denaturation at elevated temperatures, taken together with changes which have been observed in the aging dermis of the skin, suggest that the thermal denaturation of collagen may proceed at a significant rate in the living animal. That there is only limited replacement of collagen is supported by tracer experiments carried out by two groups of investigators. Their experiments will require extension but are convincing as far as they go. We will now proceed to discuss these three postulates in detail.

In any discussion of the aging of collagen, we must be careful to distinguish between maturation and senescence. The changes accompanying maturation in collagen have been described by a number of investigators, including Verzár (46), Jordan-Lloyd and Marriott (25), Sobel (42), and Banfield (4, 5).

The collagenous tissues of a young animal are rich in ground substance. The collagen is dispersed, and relatively large amounts may be extracted with saline and citrate buffers.

As an animal matures, the collagen becomes progressively more organized, and the concentration of ground substance decreases (42). As the concentration of ground substance decreases, the fractions of collagen which may be extracted in saline, phophate buffer, or citrate buffer decrease (34). The dispersed, randomly oriented molecules and microfibrils of this extractable collagen associate into larger fibrils of more definite orientation (26), as indicated by the appearance of the characteristic X-ray diffraction pattern with 640 A periodicity (13).

Once organized into larger fibrils, the collagen becomes relatively inert (32, 44). Reabsorption becomes difficult, although reabsorption definitely occurs in certain situations, such as in the uterus following pregnancy (18), in the healing of carrageen granulomas (22), and in limb atrophy (40).

Senescence is more difficult to study than maturation. In senescent humans degenerative changes in the collagen of the dermal layer of the skin have been reported by Strobel (43) and others. Evidence for degeneration during senility has been reviewed by Cooper (10).

Strobel observed that collagen fibers from the middle thirties on show increasing atrophy and degeneration. In senile elastosis, which probably represents an exaggerated form of the senile changes occurring in aging skin, the accumulation of large amounts of degenerated collagen having some of the staining characteristics of elastin has been observed (45).

When a collagenous tissue is heated in vitro,

certain changes occur which histochemically resemble those observed during senescence (3).

Can thermal changes occur in vivo? If so, they might present some serious physiologic problems.

When a tendon, which is composed of strands of oriented collagen, is heated, it becomes disorganized, and as the temperature approaches what is called the critical shrinking temperature, it contracts to about one-third of its original length.

If a band of collagen surrounding a blood vessel shrank to two-thirds of its original length, the cross sectional area of the lumen would decrease to less than 44 per cent of the original area, and the blood flow at constant pressure would decrease to less than 20 per cent of the original flow.

Using the theory of absolute reaction rates, we will now attempt to estimate the rate at which the thermal denaturation of collagen might occur in vivo at 38 C.

C. E. Weir of the United States National Bureau of Standards has made a careful study of the rate of shrinkage of tendon obtained from the tails of kangaroos (47).

The rate of thermal denaturation can be conveniently measured in the laboratory over a temperature range of approximately 7 degrees, for example, between 58 and 65 degrees. Over this temperature range the time required to attain one-half shrinkage will vary from several hours to several seconds.

Weir calculated that the heat of activation of kangaroo tail tendon was 141 kilocalories per mole, with a standard deviation of 43. He calculated the entropy of activation to be 349 calories per mole degree with a standard deviation of 0.6. Reinserting these values into Equation No. 8, we have attempted to estimate the time required for one-half shrinkage at 38 C. The results of our calculations are shown in table 1.

According to these calculations, in 215 years, the molecules of collagen in a kangaroo would undergo one-half shrinkage.

Due to the exponential nature of Equation No. 8, very small variations in the values assigned to $\triangle H$ and $\triangle S$ produce large variations in calculated shrinking times.

This is well illustrated in table 2, where the values for $\triangle H$ and $\triangle S$ obtained by Weir in 8 separate experiments are tabulated together with the values for the time of one-half shrinkage which may be derived from them. There is such a large variation in the calculated time of one-half shrinkage that little significance can be attached to any particular numerical value.

TABLE 1. THE EFFECT OF TEMPERATURE ON THE SHRINKAGE OF KANGAROO TAIL TENDON.

Temperature	Time required for one-half shrinkage seconds	or
38	6.77×10^9	215 years
43	1.89×10^8	5.77 years
48	5.41×10^6	3.58 months
53	1.77×10^5	1.92 days
58	6.45×10^3	1.79 hours
63	2.49×10^2	4.32 minutes

*Calculated from the data of C.E. Weir, J. Research Nat. Bur. Standards 42, 17 (1949) assuming that the heat of activation is 141 kilocalories per mole and the entropy of activation, 349 calories per mole degree.

Pankhurst (35) describes a phenomenon which he terms "incipient shrinkage." Air-dried collagen (source unspecified) was heated at 45 C. in atmospheres of varying humidities for periods up to 80 days. The collagen was then immersed in water and the amount of water absorbed by 100 Gm. of collagen was measured. Treatment at 45 C. decreased the ability of the collagen to absorb water. The untreated, air-dried collagen was capable of absorbing 126 Gm. of water. Collagen heated at 45 C. at 100 per cent humidity for 80 days absorbed only 75 Gm. of water. If 75 Gm. is taken as the amount of water absorbed by collagen subjected to maximum "incipient shrinkage" at 45 C., the process was 50 per cent completed in 11 days. This would indicate that denaturation measured in terms of "incipient shrinkage" occurs fairly

TABLE II. YEARS REQUIRED FOR ONE-HALF SHRINKAGE CALCULATED FROM INDIVDUAL EXPERIMENTS.

ΔH kilocal/mole	ΔS cal/mole deg.	$t_{1/2}$ 38° Years
136	334	106
136	330	84
145	361	319
168	431	2145
152	383	390
141	348	319
120	292	0.96
128	313	10.6

Calculated from data of C.C. Weir, J. Research Nat. Bur. Standards 42, 17 (1949).

rapidly at temperatures not too far removed from the physiologic range and might proceed in vivo at a finite rate.

That there is some physiologic significance to the critical shrinking temperature of collagen (although not necessarily in respect to aging) is suggested in table 3. This tabulation compares data reviewed by Brett (9) on the maximum temperature tolerated by various species of fish with the critical shrinkage temperatures of fish collagens reported by Gustavson and Takahashi (17) and tabulated by Gustavson. There is a definite correlation between the environment in which a fish lives and the critical shrinking temperature of his collagen.

TABLE III. SOME THERMAL RELATIONSHIPS BETWEEN THE COLLAGENS OF FISH AND MEN.

Species	Critical Shrinking Temperature	Lethal Temperature
Cold Water Fish	33-44	21-28
Warm Water Fish	49-58	31-37
Man	60-62	43-45

The evidence does not support an unqualified statement that senescence is accompanied by a molecular degeneration of collagen of thermal origin; however, it does suggest that this should be considered as a possibility.

We will now discuss the postulate that the rate of replacement of collagen is sufficiently slow in the mature animal to permit defective molecules to accumulate.

In the rat the replacement is so slow that it is difficult to measure. Neuberger, Perrone, and Slack (31, 32, 33) employing carbon-14 have attempted to measure the turnover of collagen in several tissues. They estimated that the half-life of rat tail tendon collagen is "about 500 days or more." Since the mean survival age of rats is only about 700 days, these results indicated that many collagen molecules persist throughout the life span of an animal.

The observations of Neuberger, Perrone, and Slack have been confirmed by Thompson and Ballou (44), who maintained rats on tritium water. In some of their experiments, rats were conceived by mothers receiving tritium water and were weaned on tritium water. These animals were taken off tritium when 6 months old. There was little decrease in the specific activity of the carcass collagen during the next 300 days, and that which occurred can be attributed to dilution of labeled collagen by unlabeled collagen during growth.

These studies suggest that humans may face the problem of maintaining their collagen in a native state for over half a century of continuous incubation at 38 C. Experiments similar to those of Neuberger, Perrone and Slack, and Thompson and Ballou would be difficult with human subjects. High levels of isotope would be required to estimate the limited collagen formation and replacement anticipated in adults. Isotopic studies utilizing tritium rather than carbon-14 might prove more practical from the viewpoint of radiation hazard. Another approach (originally suggested by Dr. W. L. Hughes of this laboratory) would be to estimate collagen turnover from the amount of natural tritium present.

Rain water is enriched with atmospheric tritium (6). This tritium should find its way via food and water into the tissues, where some should be stably bound to protein. If amino acids containing a natural abundance of tritium were incorporated into a protein such as collagen and this protein was not renewed, the tritium in the non-exchangeable hydrogens of these amino acids would decay. Tritium has a half-life of 12.5 years. If a 78-year-old man replaced no collagen after the age of 40, his non-exchangeable collagen tritium would have decayed through three half lives down to 12 per cent of its initial activity. Such a change in tritium concentration should be detectable. The principal problem in carrying out such studies is the extremely low natural abundance of tritium, which requires the counting of large samples by highly specialized counting techniques.

While collagen turnover as well as elastin turnover (41) is very slow or almost nonexistent in some tissues, other components of connective tissue may turn over quite rapidly (39). The definite differences between the connective tissue and tendon of young, mature, and senile rats (46) most certainly involve changes in the concentrations of glycoproteins and mucopolysaccharides. If in addition, there are changes within the collagen molecules, such changes must be considered as part of a complex interrelationship existing between the various chemical and cellular components of connective tissue during maturation and senescence.

DESOXYRIBONUCLEIC ACID

There are a number of reasons for suspecting that the aging of a cell is associated with aging of genetic material.

The extremely low turnover of desoxyribonucleic acid (DNA) found in tracer experiments in resting cells would suggest that the resting cell has a very limited capacity to synthesize new genetic material (15, 20, 21, 38) and that DNA is synthesized only during mitosis. If DNA is responsible for the synthesis of ribonucleic acid (RNA), which in turn is responsible for the synthesis of protein, then gradual alteration of genetic material would reduce the vigor and vitality of cells. They would become old.

A very limited rate of mitosis is found in many tissues (28). No mitosis can be detected in nervous tissue and in the sense organs after birth. After the cessation of growth, mitosis ceases or is very limited in smooth, striated, and cardiac muscle, exocrine and endocrine glands, cartilage, bone, liver, and kidney. In such resting cells, it is doubtful whether there is any appreciable replacement of DNA. Hypertrophied and hyperchromatic nuclei have been observed in aging mammalian cells, such as are found in liver and cardiac muscle which cease to divide in the mature animal (11).

In the section on collagen we were dealing with fairly well defined chemical entities. This is not the case with the gene, in situ in the chromosome. The relationship of DNA, nuclear protein, and genes is a matter of conjecture, although ultimately genetic specificity is thought to reside in DNA.

What may be postulated concerning the stability of genes incubated at 38 C. for the life span of an animal?

Data from the literature on several different kinds of materials will be discussed. The materials include calf thymus nucleic acid, the purified transforming factor of *Hemophilus influenzae*, and phage, plant, and animal viruses.

A number of thermal studies have been made on purified calf thymus nucleic acid. The most common criterion for change is a decrease in viscosity. There are large variations in the data reported in the literature for the heats of activation. The most recent data would indicate that solutions of mammalian DNA properly prepared may show great thermal stability. Doty and Rice (12) found that their most stable preparations maintained an intrinsic viscosity (measured at 25 C. and extrapolated to zero gradient) of 72, when heated for one hour up to a temperature of 85 C. It is, however, practically impossible to relate changes in the viscosity of DNA to functional changes in genes.

On the other hand, there are certain materials and organisms which have many of the characteristics of genes and which may be isolated in a fairly high state of purity. A transforming factor for *Hemophilus influenzae* has been prepared by Zamenhof, Alexander, and Leidy (49) which has the property of changing *Hemophilus influenzae* Type A into Type B.

This material appears to be pure DNA. Zamenhof (49) found that a 20 per cent decrease in viscosity of these solutions was accompanied by a 90 per cent decrease in transforming ability. This material was also found to be quite stable at elevated temperatures. Zamenhof did not detect any inactivation in one hour at 80 C., although inactivation went rapidly at higher temperatures. Material of this stability would not undergo appreciable thermal change at 38 C.

Viruses by analogy seem to resemble chromosomes in regard to their ability to reduplicate themselves within the cells of the host and bear at least a superficial resemblance in chemical composition. The thermal stability of phages, plant viruses, and animal viruses might conceivably be of the same order as that of genetic material.

Values for $\triangle H$ and $\triangle S$ for a number of viruses calculated and tabulated by Pollard (36) from data collected both in his own and other laboratories, are presented in table 4, together with our own calculations from the data of Zamenhof. In the last column, we have calculated, assuming the validity of the theory of absolute reaction rates, the life of such material at 38 C. This is expressed in terms of the time that would be required for 1 per cent inactivation rather than the time required for 50 per cent inactivation. The mammalian cell is probably genetically more complex than a virus, so that a smaller fraction of chromosomal loss would impair the function of the cell.

There is a tremendous spread to the $t_{0.01}$ values listed in the table, and they extend in both directions beyond the life spans of any living organisms.

In instances where a virus or phage is extremely susceptible to heat, as in the case of influenza virus, snap bean mosaic virus, M-1,

TABLE IV. THE THERMAL STABILITY OF A NUMBER OF VIRUSES.

Virus	ΔH calories per mole	ΔS calories/mole degree	Years for 1% inactivation at 38°
E. coli phage'			
T - 1	95,000	207	260
T - 2	71,700	139	.47
T - 3	105,000	246	1.28
T - 5	82,000	165	730
T - 7	60,700	114	9.4
B. Megaterium phage			
M - 1	76,000	165	1.15×10^{-3}
M - 2	82,000	183	.023
M - 3	87,000	195	.063
M - 4	136,000	347	1.28
M - 5	112,000	254	3810
Tobacco mosaic	40,000	18	6850
Southern bean mosaic	25,000	-5	2.10×10^{-2}
Influenza	34,000	39	1.56×10^{-5}
Hemophilus influenzae transforming factor	129,529	289	2.27×10^{8}

M-2, and M-3 phage, the rapid inactivation is probably associated with the inactivation of proteins associated with infectivity rather than with inactivation of DNA. The rather striking effect of Mg^{++} and other ions on the thermal stability of phage noted by Adams (1) and Friedman (14) is another variable which markedly influences such a series of measurements.

On the other hand, it is conceivable that the thermal stability of M-4 *B. megaterium* phage or T-7 *E. coli* phage resembles that of the genetic material in a mammalian chromosome. The very great thermal stability of *Hemophilus influenzae* transforming factor which is the most pertinent example, however, indicates that certain forms of DNA have a stability considerably in excess of that required to survive the lifetime of an animal at 38 C. Therefore, no real conclusion is justified until more information is available on the structure of genes.

HEMOGLOBIN

Most globular proteins of the body are continuously replaced. Their lives are shorter than the life spans of the cells in which they are produced. Hemoglobin is a special case. The theory of absolute reaction rates may be used to estimate the time required to denature 50 per cent of the hemoglobin in a red blood cell at 38 C. Johnson, Eyring, and Polissar (24) give values for $\triangle H$ of 76,300 cal./mole and for $\triangle S$, of 152 cal./mole degree. These values were calculated from the data of Lewis (29). The measurements were made on 1 per cent solutions of crystallized oxyhemoglobin from beef blood, both in water and in 0.15 N ammonium sulphate at pH 6.8. Insolubility was taken as the criterion of denaturation. Measurements were made at 60.5, 64.0, and 68.0 degrees. If the calculated values for $\triangle H$ and $\triangle S$ are reinserted into Equation No. 8 for 38 C., the time which would elapse before 50 per cent of the hemoglobin became denatured is found to be 8.7 months.

Mammalian red blood cells have definite life expectancies (30). While we are not aware of any determinations of the life expectancy of beef red blood cells, it is probably of the same order as for human red blood cells, namely, 4 months. It is therefore possible that the spontaneous thermal denaturation of hemoglobin may play a role in determining the life span of the red blood cell.

The observations of Bernard, Dantchev, and Gajdos (7) suggest that changes do occur in the

hemoglobin of maturing red cells, newly formed rabbit hemoglobin being more resistant to denaturation with alkali.

On the other hand, there is no evidence that denatured hemoglobin accumulates in the red cell population. If hemoglobin were denatured with a t ½ of 8.7 months, the steady-state concentration of denatured hemoglobin assuming a 4-month life for the red blood cell would be 14 per cent. There certainly is no accumulation of denatured hemoglobin or of methemoglobin of this magnitude. The concentration of methemoglobin is maintained at around 1 per cent by methemoglobin reductase. An elevation in methemoglobin concentration to higher levels is felt to be detrimental to the life of the cell (37). This is borne out by the hemolysis observed in methemoglobinemia and in stored blood in which methemoglobin accumulates.

AGING AND RADIATION

The hypothesis that aging results from the disorganization of genetic material has been advanced recently by several workers interested in the mathematical relationships between radiation injury and survival, most forcibly by Yockey (48). Blair (8) has observed that whole-body radiation will alter the survival of an animal population and accelerate some of the changes associated with physiologic aging. Yockey's suggestion based on information theory that aging results from a loss of genetic specificity is extremely attractive. At the moment it is not easy to reconcile with the limited information which indicates an appreciable thermal stability of DNA. On the other hand, very small changes in the chemical structure of DNA might alter the specificity of the genetic information it transmits.

It is probable that the consequences of nuclear damage in the living animal predominate over direct chemical effects of radiation on substances such as collagen where we have found that 500,000 r of cobalt-60 gamma radiation is required to lower the critical shrinking temperature 1 C. In the case of collagen we would anticipate that the organic matrix of the ground substance, the glycoprotein, and the mucopolysaccharides which the studies of Schiller and associates (39) indicate are in a continuous state of flux, would be affected by a much lower dose of radiation than the collagen molecules themselves.

Premature maturation in connective tissue following irradiation might follow a decreased synthesis of ground substance due to nuclear injury and death of fibroblasts, permitting the diffuse collagen of the young animal to organize into dense fibrous collagen.

Quantitative measurements of the changes in collagenous tissue during maturation and senescence such as those of Verzár (46) on the rate, degree, and force of shrinkage of rat tail tendons may prove extremely useful in deciding whether or not the decreased longevity observed following radiation may be attributed to accelerated maturation and senescence.

SUMMARY

Macromolecules such as proteins and nucleic acids are susceptible to thermal denaturation. The rate at which this denaturation might be expected to occur may be predicted from the theory of absolute reaction rates. An attempt has been made to estimate the thermal stability of collagen, desoxyribonucleic acid, and hemoglobin.

Evidence is reviewed which would indicate that the collagen of connective tissue, bone, tendon, and blood vessels may alter and contract with age due to thermal denaturation. Changes occur in collagen during senescence which resemble those produced in vitro by heat. Extrapolation of physical-chemical measurements made at elevated temperatures to 38 C. suggest that thermal changes may occur in vivo at 38 C.

The changes which occur in connective tissues during maturation are accompanied by decreased deposition of ground substance and decreased synthesis of dispersed collagen. The decrease in cellular activity observed during maturation and senescence may resemble in certain features the decrease in cellular vitality and survival following radiation injury. In both instances cells may lose vitality and die due to injury to genetic material responsible for cell duplication and enzymatic specificity.

The theory of absolute reaction rates cannot be used to predict the thermal stability of genetic material at 38 C. because of the lack of information about its chemical structure and the relationship between chemical structure and function.

Quantitative measurements of the changes occurring in collagenous tissue during maturation and senescence should prove very valuable in establishing whether or not there is a rela-

tionship between the decreased longevity observed following radiation and physiologic aging.

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ATHEROSCLEROSIS: A HYPOTHESIS CONCERNING THE INITIATING STEPS IN PATHOGENESIS

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The rate of progression of atherosclerosis as manifested by coronary artery disease is correlated with the serum levels of low density lipoproteins (11). What may be the basis for this relationship? It is suggested that this correlation exists because the concentration of the material involved in the initiation steps in atherosclerosis parallels that of the low density lipoprotein. It is further suggested that this material is produced in the lipoprotein through oxidative-polymerization of some of its lipid constituents.

From the general character of oxidation reactions involving molecular oxygen and the ready oxidation of fats by oxygen (17) it might be expected that some of the lipids in the serum lipoproteins would undergo oxidative polymerization to higher molecular weight materials containing one or more groups such as hydroxyl, peroxy, carbonyl, or carboxyl. The type of product produced and the rate of formation would depend on such factors as the following: the concentration and types of cholesterol esters, detergents (such as the phospholipids and the globulins), oxidation catalysts (iron, copper, cobalt, for example), oxidation inhibitors (such as mercaptans, vitamin K, and vitamin E), fats liberated into the blood stream from the fat depots, and ingested fat. In addition, hydrolysis of ester groups of oxidized-polymerized material would yield compounds containing hydroxy and carboxyl groups. Some of the data which support this concept of the in vivo oxidation of fats to form higher molecular weight compounds follow: Fats have been shown to be oxidized by molecular oxygen in vitro at body temperature and the reaction is catalyzed by such common metallo-organics as cytochrome-C, catalase, and hemoglobin (32). Hemin catalyses the autoxidation of linoleic acid in heterogeneous emulsion (16); the catalysis is stopped by the addition of acetic acid, dioxane, pyridine, alkali or bile which bring about homogenization of the system—that is, this oxidation reaction occurs at a measurable rate only in the dispersed linoleic acid phase. It is well known that some fats can be oxidized to high molecular weight compounds at ambient temperatures; the drying of paints is an example.

It has been shown that atherosclerotic aortae contain lipoperoxides and that the content of the peroxides parallels the degree of severity of the atherosclerosis while normal aortae are free of lipoperoxides (8). Such peroxides are produced in the oxidation of fats. The following is an excerpt from the paper which reported this study:

"It may be that the peroxidation is secondary to the deposition of lipids, but the irreversible nature of the formation of lipoperoxides and the ability of such compounds to form a variety of secondary products suggests the possibility that an active part is played by these compounds in the pathogenesis of atherosclerosis."

Compounds that can act as oxidation inhibitors, such as ascorbic acid, thiamine, iodide (22), and thyroxine (10), in the diet of rabbits being fed cholesterol, decreases the severity of the cholesterol-induced atherosclerosis. Futher, a mixture of vitamin A palmitate and α-tocopherol acetate, fed orally, very significantly reduced plaque formation, aortic total fat, and free and esterified cholesterol in chickens on a regular diet (34).

Pyridoxine deficiency favors development of atherosclerosis (14, 28, 31). Is this because pyridoxine, a good heavy metal chelating agent, decreases the effective concentration in the serum of metal oxidation catalysts?

Atherosclerosis is a prominent feature of chronic disease states associated with an elevated cholesterol blood level (10). This is not surprising from the point of view of the present theory, since cholesterol is easily oxidized by molecular oxygen (26) and about two-thirds of the cholesterol in the serum lipoproteins occurs as esters of fatty acids (10, 12) (these are chiefly unsaturated fatty acids whose ready oxidation is well known).

Studies of the degradation of isolated lipoproteins (both human and rabbit) indicated that the observed changes were due to oxidation (29):

The changes noted on dialysis against a large volume of buffered NaCl solution or ordinary water, as observed by ultracentrifugal analysis were: 1) The heterogeneity of the lipoprotein material increased, as evidenced by the spreading of the peak; 2) Low density material of lipid nature appeared—this rose rapidly to the meniscus and tended to form a compact layer; 3) The concentration under the lipoprotein peak diminished and there was an increased amount of material which sedimented under the experimental condition; 4) The rate of flotation of the lipoprotein peak progressively decreased. In addition there frequently developed a visible turbidity due to aggregation of lipid-like material mentioned above in second instance. If all traces of cupric ion (less than circa 0.1 ppm) were removed from the environment the lipoprotein was found to be stable upon dialysis; numerous other metal ions were without effect. Several substances were found which would stabilize the lipoprotein—these were antioxidants or else complexing agents for copper. It was concluded that the data supported the view that the degradative process was oxidative in nature and was catalyzed by copper.

In addition, changes were produced in lipoprotein concentration similar to the changes resulting from dialysis by the addition of 1 per cent hydrogen peroxide and a trace of cupric ion (cupric ion decomposes hydrogen peroxide to water and oxygen via a free radical reaction involving hydroxy radicals) (33). When cupric ion was not present or when it was complexed with Versene, the peroxide did not seem to affect the lipoprotein.

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Thus, oxidation of some of the constituents of the lipoproteins may contribute to the formation of lower groups from the higher S_f groups. Hydrolysis of glyceryl esters of lipoproteins also results in formation of lower S_f molecules from higher ones (13).

The foregoing data on oxidation taken as a whole suggest that oxidative-polymerization proceeds in the lipoprotein in vivo and that this process is analogous to that occurring in simple emulsion polymerization systems (5).

There is a good correlation between the steady state concentration of low density lipoproteins of the S_f 0-400 group and the extent of atherosclerosis as manifested by coronary artery disease. Hence, if oxidized-polymerized lipids are the initiating agents in atherosclerosis, their steady state concentration must parallel that of these low density lipoproteins. This does not mean that lipid oxidation-polymerization does not occur elsewhere than in members of the S_f 0-400 group, but implies that if it does, the oxidized material is formed in relatively small amounts or eventually comes to be carried by lipoproteins of the S_f 0-400 range—for example, oxidation of lipid might occur in a lipoprotein of $S_f > 400$, but as indicated above, this tends to convert it into one of lower S_f.

Tissue response to foreign material in the arterial wall probably plays a part in the development of human atherosclerosis as shown by experiments in which lesions were produced in arteries of dogs by injecting into the media human fat alone or mixed with fat acids, calcium soaps or cholesterol (4). The severity and chronicity of the lesions varied with the acidity and speed of dispersal of the fat mixture. Human fat and fatty acids produced marked inflammatory lesions, which healed rapidly because the lipids were absorbed readily. Human fat mixed with calcium soaps or cholesterol was absorbed slowly and caused chronic lesions. Repeated intravenous injections of solutions of large molecules such as polyvinyl alcohol or sodium cellulose glycolate produced atheromatosis in the arteries of dogs (18, 19).

Thus, it would seem that both the type of molecule entering the arterial wall and the length of time it stays there are involved in the development of atherosclerosis. In the case of human atherosclerosis, the types of molecules involved in pathogenesis are apparently primarily some lipids associated with the lipoproteins of the S_f 0-400 class. The time that these lipids—presumably those which have undergone oxidative-polymerization—remain in the arterial wall is a function of both the size of the molecules (and their chemical nature) and the degree of interaction between the lipids and the constituents of the arterial wall. Interaction would range from relatively slight (as between molecules of the arterial wall and lipids containing comparatively non-polar groups such as carbonyl, hydroxyl, and esters) to quite strong, as in the formation of mixed calcium salts between carboxyl groups of the lipid and those on arterial wall constituents.

Hence, for a given size and type of lipid, other conditions being constant, the rate of passage through the arterial wall, and hence the time spent in the wall, is a function both of the chemical character of the lipid and that of the arterial wall. What determines the contribution of the arterial wall to the anchoring of the lipid molecule? According to a recently proposed theory of aging (which relates aging to the deleterious side effects of free radicals normally produced in the body) a slow net oxidation of the constitutents of the body would be expected, including the connective tissues of the large vessels such as the aorta (15). Further, it would be anticipated that carboxyl groups would be among the oxidized groups formed (on the side chains of proteins, for example) and that these in turn would form insoluble calcium salts. Thus, an increase in the calcium content of the tissues with increasing age would be expected. It has been known for a long time that the calcium content of the tissues of the body increases with age. Further, it has been shown that the content of aspartic and glutamic acid (both dibasic acids) of the elastin of the aorta increases with age, and it has been suggested that the increasing calcium content of the aorta is related to this fact (21). Thus, the ability of the arterial wall to anchor material such as oxidized lipids would be expected to increase with age through the formation of polar groups on the constituents of the wall. That arterial age is a factor in the development of atherosclerosis was indicated by the experiment (27) in which, under analogous experimental conditions, rabbits one year of age responded to cholesterol feeding with atherosclerosis, whereas 5, 10, and 25 week old animals did not. All rabbits, however, regardless of age, reacted with hypercholesterolemia of almost equal degree.

In addition to the interaction of the oxidized-polymerized lipid with fixed tissues of the arterial wall, such as elastin, there may also be one involving the cells, which may contribute to the fixation of the lipid material.

The expected effect of age on the contribution of the arterial wall to the rate of progression of atherosclerosis is paralleled by an increase in the level of the low density lipoproteins. This increase with time (over the major portion of the life span) follows the same function as does the rate of aging, as measured by the mortality rate.* That is, the rate of increase at any given time is proportional to the lipoprotein level, i.e. $\frac{dS_f}{dt} = kS_f$ or $\log S_f = kt + c$, where c is a constant of integration. Thus, the lipoprotein levels appear to reflect the rate at which the body as a whole is aging. For females the equation is good from age 25 to 70 years, while for males it is valid between ages 15 and 35. The slope of the curve log S_f—age is essentially the same for both males and females below age 35; above age 35, when S_f 0-400 is circa 600 mg% (9), the curve for males begins to bend downwards. Recently it has been shown that the lipoprotein values also decrease, from a maximum S_f 0-400 of circa 700 mg%, with advancing age in women past 70 (6). Consideration of the data for abnormal lipoprotein states (10, 30) indicates that this phenomenon is due, at least in part, to saturation of the β-globulins, normally associated with the low density lipoprotein, with lipid.

The fact that a maximum level of S_f 0-400 is attained eventually with increasing age does not necessarily mean that the steady state concentration of the oxidized-polymerized lipid assumed to be responsible for initiating atherosclerosis does not continue to rise un-

* Calculated from data in the paper by Glazier, F. W., and Associates. Human Serum Lipoprotein Concentrations. *J. Gerontol.*, 9: 395-403, 1954.

abated, but rather that when the Sf 0-400 lipoproteins are saturated the excess oxidized-polymerized molecules are carried elsewhere—probably in the higher S_f groups (or in higher concentration in the S_f 0-400 or both).

Thus, atherosclerosis appears to be the result of three primary processes: 1) Oxidative-polymerization of constituents of serum lipoproteins; 2) Anchoring of the oxidized material in the arterial wall; 3) An inflammatory reaction induced in the arterial wall by these oxidation products. With increasing age the contributions of the first two processes increase while that of the latter decreases. Hence, it is conceivable that a point might be reached where the rate of progression of atherosclerosis might decrease with age in spite of increasing contribution of the first two primary processes. In addition to age, a number of other modifying factors are superimposed on the primary processes (1, 2, 3, 7, 20, 23, 24, 25, 35)—their influence seems to be best understood when considered from the standpoint of their effect on the suggested primary processes.

This concept of the pathogenesis of atherosclerosis is suggestive of means to slow down the process. One of the most obvious is to decrease the chain length of the oxidative-polymerization reactions by increasing the serum concentration of compounds capable of acting as oxidative inhibitors. A study of the oxidative characteristics of lipoproteins formed on different dietary regimens may be quite productive. This may be particularly helpful in understanding why increasing the unsaturation of dietary lipids tends to decrease cholesterol and lipoprotein levels.

SUMMARY

A hypothesis has been developed concerning the initiating steps in atherosclerosis. These postulated steps are: 1) Oxidative-polymerization of constituents of serum lipoproteins; 2) Anchoring of the oxidized materials in the arterial wall; and 3) An inflammatory reaction induced in the arterial wall by these condensed products. With increasing age the contribution of the first two processes increases while that of the latter decreases. This hypothesis is suggestive of means whereby the rate of progression of atherosclerosis may be inhibited.

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GERIATRIC PRACTICE

BENIGN PROSTATIC HYPERTROPHY

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One of the most frequent GU problems confronting the middle-aged and old man is benign prostatic hypertrophy. Other names for the same condition are prostatic adenoma, benign enlargement of the prostate, and prostatism. Most frequently the condition makes its appearance after the age of 50, and about 40 per cent of males over this age show some evidence of it.

The etiology is unknown although there must be some relationship between prostatic hypertrophy and altered relationships of the sex hormones at this age. A eunuch has never been known to develop BPH and, in fact, castration after the adenoma develops frequently causes the tumor to shrink in size similar to the malignant prostate after removal of the testicles. I am not suggesting this as a treatment for benign prostatic hypertrophy, however, because we have less mutilating procedures than castration to control the obstructing adenoma.

Pathologically, the disease is a true tumor of the prostate which develops in one or several of the prostatic lobes at the same time. There may be enlargement of the lateral lobes with encroachment on the prostatic urethra, or there may be enlargement of the middle lobe which then projects into the bladder, and may act as a ball valve at the bladder neck. There may also be a collar type of hypertrophy which has uniform growth entirely around the vesicle neck. There can be a combination of these in any one patient.

The adenoma develops, and in doing so, compresses the normal prostate between itself and the anatomic capsule of the prostate. The so-called surgical capsule of the prostate actually is the anatomic capsule plus the compressed but otherwise normal prostate lying to the periphery of the prostatic adenoma. The normal prostate weighs about 20 Gm. The average enlargements seldom exceed 80-90 Gm., but there have been reported cases in which 300-400 Gm. were removed.

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Microscopically, the picture is one of hyperplasia of the glandular epithelium with marked increase in the connective tissue stroma. Hypertrophy is then a misnomer, and hyperplasia would be a better term.

The symptoms of prostatic hypertrophy vary considerably from no genito-urinary symptoms at all to sudden acute retention with the inability to pass any urine. However, the most common symptom is frequency of urination, either diurnal or nocturnal (nocturia) with gradual increasing severity until the patient has to urinate perhaps 10-15 times each day and as often as 4-6 times at night. Along with the frequency is usually some degree of urgency making it impossible for the patient to exercise any degree of control over his voidings. The urinary stream becomes smaller in both size and force, and terminal dribbling is usual. Most patients with any degree of hypertrophy, and especially if they are carrying any residual urine, will comment that even after voiding all they can the bladder does not feel empty.

If the residual is large enough, there may be evidence of lower abdominal enlargement, and percussion and palpation will reveal an enlarged bladder. Pressure over the suprapubic area will cause the patient to feel as though he must urinate. Other symptoms and signs of prostatic enlargement are bleeding from the enlarged, congested prostate. This may be quite profuse and require surgical intervention to control, although usually an indwelling bag catheter is sufficient. Infection may complicate the picture, especially if the bladder is holding any residual. Stagnant urine offers one of the best natural occurring culture medias in the human body. Again, an indwelling catheter will control this problem until definitive surgery can be carried out.

The complications of BPH are many. Foremost, of course, is the increased intravesical pressure necessary to evacuate the bladder, however ineffectively. The bladder musculature hypertrophies from overwork, producing visible enlargement of the muscle bundles, which are are called trabeculations. Between

these trabeculations the bladder mucosa lies in a shallow depression which gradually deepens, producing a "cellule" and eventually a "diverticulum." In some instances, the increased intravesical pressure is transmitted to the kidneys and ureters, producing obstruction to the flow of urine to the bladder. The hydronephrosis and hydro-ureter which result lead to infection and reduced renal function. Occasionally the uretero-vesical valves become incompetent, allowing the urine to back up in the ureters when the patient attempts to void, a condition called reflux. This too predisposes to increased infection and loss of renal function. The constant presence of infected residual urine may also produce a bladder stone, with a marked increase in symptoms caused by the irritative qualities of the stone. Last, but by no means least, is the complication of acute retention to which all these patients are susceptible. Many times the very first symptom the patient is aware of is the episode of inability to urinate at all. In this event of acute retention an indwelling catheter is put into the bladder and then a complete urologic investigation carried out.

An examination of the patient who presents himself to his physician with any of the aforementioned symptoms then must be carried out to determine if he has benign prostatic hypertrophy or some other urologic problem. Of course, the very first step in the procedure is a careful and detailed urologic history outlining how long symptoms have been present, exactly what they are, and their sequence of occurrence. Following the history, next in order is the physical examination, which should include not only a careful examination of the abdomen and renal areas, but also examination of the genitalia, and the prostate by means of a rectal examination. It is wise to have the patient urinate into two glasses *before* examining the prostate so that if the prostatic secretion is heavily infected it will not contaminate the urine specimen; after a massage of the prostate such urine examination is not possible. Therefore, after the physical examination, but before the prostate is massaged, the patient should be asked to urinate into two glasses, and it should be noted at this time whether there is any difference in the appearance of the urine samples in the two glasses, whether there are any shreds or cloudiness in the first or in the second, or blood. At this time, too, can be noted the exact character of the urinary stream. Rectal examination of the benign prostate reveals that the gland is much larger than normal in most cases. Its surface is smooth, and the landmarks of the prostate may be missing. The median furrow may be completely absent and the lateral sulci are extremely deep. The consistency of the gland is best described as rubbery, and on many occasions, it is almost impossible to reach over the gland to palpate the bladder and the seminal vesicles.

Following the routine history and physical examination, it is my custom to obtain an intravenous PSP test, which is done for two reasons. First, it gives some indication of the patient's renal function, and second, it is useful in determining in a rough way how much residual urine he is carrying. This procedure should be carried out as an office procedure at the time of his first office visit. One cc. of PSP is given intravenously, the patient having completely emptied his bladder prior to the beginning of the test. I usually collect a single specimen at 1 hour and 10 min., during which time the patient is allowed to have fluid ad lib. At this time a normal specimen will range somewhere between 46-60 per cent excretion of the dye, and we infer two things if it is within this range. First, that the patient's renal function, at least his tubular function, is within normal limits, and secondly that he is probably almost completely emptying his bladder, since if very much was retained, the specimen would be low. If the percentage of dye excreted is low, at this point we generally pass a small Coude catheter gently into the bladder to determine the exact amount of the residual urine.

Intravenous urograms are obtained on all patients who are suspected of having BPH, not only to determine the status of the upper tracts, but also to determine the presence or absence of any complications in the bladder. The x-ray studies of the urinary tract will show to some extent the function of the kidneys, whether there is any dilatation present, the presence or absence of stones, not only in the kidney but also in the bladder, and while the excretory cystogram is notoriously inaccurate, it will, in some cases, give an indication as to the prostatic size. One other x-ray study of the urinary tract is usually carried out, a retrograde cystogram, which is easily performed by filling the bladder with some radio-opaque substance, such

as 5 per cent sodium iodide or dilute solutions of the organic iodines used in obtaining a retrograde pyelogram and intravenous pyelogram. The bladder is allowed to fill by gravity to its maximum capacity, and then AP right and left oblique films are made followed by an evacuation film. This series of films will give an accurate delineation of the bladder shadow, will show the presence or absence of any diverticuli, and will give an accurate picture of the prostatic size, particularly on the oblique films. This study has the added advantage of showing the presence of any reflux which might occur up the ureters, showing decompensation of the ureteral vesical junction. It is for this reason alone that a cystogram is never carried out on the same day, or immediately following an intravenous pyelogram or retrograde pyelogram, because frequently the opaque medium used in these studies is retained in the kidneys and ureters for some time after the procedure is carried out. It would be impossible to determine whether there was reflux present unless the upper urinary tract was completely free of opaque media before beginning the cystogram.

Cystoscopy is generally indicated in the patient in whom benign prostatic hypertrophy is a problem. However, the patients must be selected, and frequently the best place to carry this procedure out is in the hospital where preparations for surgery have already been made. This procedure allows a direct view of the entire bladder wall and the prostatic urethra. Gentleness cannot be stressed too greatly in carrying out this study. Even in the best of hands the cystoscope can be a dangerous instrument. Cystoscopy in a patient with a large prostate can cause acute retention, severe infection, and sometimes uncontrolled bleeding from the prostate. In all such patients, and especially when the procedure is carried out as an out-patient procedure, facilities should be available to hospitalize the patient immediately if it becomes necessary. In some cases the correct surgical judgment would be to cystoscope the patient only after he is already under an anesthetic just prior to surgery on his prostate in order to prevent just such complications as I have mentioned.

The final decision as to whether any particular prostate should be operated on depends on many factors. First and foremost is the amount of residual urine the patient is carrying. We generally believe that any residual urine over and above 3 oz. represents a definite indication to prostatic surgery. Patients with less than 3 oz. will have to be watched closely, usually at 6 month intervals, and if their residual urine is increasing, we then recommend surgery. There are other groups of patients, however, that will require some sort of prostatic surgery even though their residual may not be enough by itself to justify an operation. These are the patients whose symptoms have become almost intolerable even though they are actually able to empty their bladder. Such patients may be getting up numerous times at night, their stream may be very small and weak and considerable straining and effort on their part may be required to evacuate the bladder. If this picture is complicated by some form of cardiovascular disease, as is often the situation with many of these old men, it is frequently possible to relieve many of their cardiovascular problems merely by allowing them to get a good night's sleep and to empty the bladder without straining. More than once we have seen a man's cardiac status improve markedly after prostatic surgery. Other indications for prostate surgery are the occurrence of various complications to the benign prostatic hypertrophy, such as a stone in the bladder, hydronephrosis, and diverticulum of the bladder. These signs all indicate that the bladder neck obstruction is severe enough to warrant intervention.

The actual preparation for surgery, once it has been decided upon, depends somewhat on the surgical risk involved. If the patient is relatively young, with a good cardiovascular system, the preparation need be no more than that for routine surgical procedures. However, in certain older individuals who are not a good risk, it may be wise to drain the bladder with continuous drainage by means of an indwelling catheter for some time before institution of prostatic surgery, particularly if the blood urea nitrogen is elevated. For many years it has been the policy of urologists to institute constant drainage in a man whose NPN or BUN was elevated, and to allow him to remain on continuous drainage until the nitrogen is stabilized, that is, reaches a constant value after continuous drainage. This does not necessarily mean a normal value, but rather the lowest possible value for this particular patient. This drainage may take several weeks or as long as a year or so before the prostatic surgery is

carried out. An indwelliing urethral catheter is extremely irritating over any but the shortest period of time, and it is therefore wise, if continuous drainage is planned for more than a month or two, to insert a suprapubic tube for the drainage of the bladder. This can be done very easily under local anesthesia if necessary, and there is hardly any operative risk connected with such a procedure. I do not recommend any sort of punch cystotomy because of the many complications which could occur from such a procedure.

The actual surgery upon the prostate gland is at present confined to four major surgical procedures. The first I wish to mention briefly is the suprapubic approach of which there are really two varieties. The first is the classic suprapubic prostatectomy which is done in two stages. The first stage has already been mentioned, that is the placing of a suprapubic tube in the bladder, through a small incision. The patient is allowed to have constant draining of the urinary tract for a period varying from a week to a month, which permits his urinary tract to stabilize. Following this, a suprapubic prostatectomy is carried out through the same suprapubic incision. This, of course, is the safest procedure and one which can be tolerated by even the poorest of surgical risks. The other type of suprapubic procedure is the one-stage suprapubic prostatectomy with or without suprapubic drainage following the surgery. Because of the newer surgical adjuncts and improved technique, this is the commonest suprapubic operation at the present time. The suprapubic operation, whether a classic or a one-stage procedure, is the operation of choice for the casual urologic surgeon because it offers less difficulty both to the surgeon and patient. The two primary complications to this procedure are 1) bleeding at the time of surgery, which can be extremely profuse and may even require packing of the prostatic fossa, and 2) obstruction at the region of the internal sphincter following surgery, which is due to the contracture of the scar tissue at the vesicle neck. This can be prevented by taking a wedge of tissue from the posterior lip of the bladder neck at the time of the prostatectomy, which allows a wide open fossa and bladder to remain.

The perineal approach to the prostate was first devised and popularized by Dr. Young of Johns Hopkins Hospital and since been advocated by Dr. Elmer Belt of Los Angeles and others. It is an ideal surgical approach to the prostate provided the gland is fairly low lying and the ischeal spines are wide spread so that adequate exposure is easily obtained. This operation is particularly suited to the older age group who are poor surgical risks since the post-operative morbidity is minimal and this type of surgery allows for the removal of fairly large glands which would be difficult to remove by any other method. The drawbacks to the use of the perineal prostatectomy are 1) the risk of incontinence because of damage to the sphincter which, while minimal, is a constant threat, and 2) it requires a very special training and technique to carry out the procedure since there is no place where a poorly trained surgeon can get into more difficulty.

The retropubic approach to the prostate, devised by Terence Millen of England, has become quite popular during the past few years. By its name one realizes that the approach is through the anterior surface of the prostatic capsule with exposure of the adenoma and its enucleation thereby. Technically, the operation is not particularly difficult, and it lends itself well to the removal of the benign prostate. The complications which might arise from the use of this particular procedure are 1) due to the fact that the bladder cannot be adequately inspected during a classic retropubic operation and 2) considerable bleeding may be encountered as one goes through the plexus of veins which lies on the anterior surface of the prostatic capsule. This bleeding can be rather profuse since the walls of these veins are very thin and friable. Other than these two problems, however, the same post-operative care can be given to the patient who has had a retropubic prostatectomy as to the one who has had a suprapubic prostatectomy. The advantages to the retropubic prostatectomy seem to be that the patients have little morbidity during the post-operative period, and as a whole, leave the hospital sooner than the other open type operations.

The last type of surgery that I wish to discuss is that of the transurethral resection which has become extremely popular with the urologic profession since 1935. This operation is the most difficult to do technically and requires the most experience on the part of the surgeon. It is designed at the present time primarily for two type of glands, the small, fibrous gland, and the carcinoma. It is my opinion that re-

sections should be reserved for those prostates smaller than 40 Gm. even though many men in the country today routinely do transurethral resections of benign prostates weighing up to and including 100 Gm. I have always considered that this is nothing more than a "surgical show." The patients with large benign prostates do better with open surgery than they do with transurethral resections.

There are complications, of course, to transurethral surgery peculiar to this particular type of operation. Lower nephron nephrosis or acute renal insufficiency develops more frequently after transurethral resection than after any other type of prostatic surgery. This occurs because of the irrigating solution used at the time of operation entering the venous channels of the prostatic capsule and being forced into the blood stream because of the hydrostatic pressure of the irrigating fluid. At the present time there are many solutions being recommended for use, and all have their advantages. In my own particular group, we are still using distilled water, and have had no trouble with it.

There are two complications of transurethral surgery which must be kept in mind and which are actually contraindications for this type of operation in some cases. Foremost of these is continued infection following the surgery caused by dead or devitalized tissue still remaining in the prostatic fossa due to an incomplete resection. Such tissue will cause a severe infection to be maintained and will actually give the patient considerable symptoms; until it is removed transurethrally, he will get no better. The other problem is bleeding from the prostatic urethra, months or sometime years, following the original surgery, a condition which is not seen frequently with other types of prostatic surgery. In the younger man who has an enlarged, benign prostate, it is sometimes better to elect to do an open operation with removal of all of the adenoma, rather than to perform a transurethral resection with the resultant risk of regrowth of the adenoma or of leaving some behind.

No discussion of benign prostatic hypertrophy would be complete without at least mentioning the more or less parallel urologic problem, that of prostatic carcinoma. It has long been recognized that the only real cure for prostatic carcinoma is the early recognition and removal of the small, hard nodule while it is still confined to the prostate and its capsule. It has been the experience of urologists all over the country that less than 5 per cent of all prostatic carcinomas will fall into this early group. It is possible to have carcinoma of the prostate occurring concomittently with a benign adenoma and merely be represented by a hard small nodule in the midst of a large rubbery benign gland. An open perineal biopsy should be performed on such lesions, of course, as soon as discovered. The various closed types of biopsy that have been recommended over the years should be mentioned only to be condemned because, while a positive biopsy leaves no question as to the nature of the lesion, the number of false negatives might be quite large in a good sized series. It has, therefore, been our practice to biopsy a nodule through an open perineal biopsy. We believe, too, that frozen sections of prostatic tissue can be fairly misleading even in the hands of the best pathologist, so that we have taken the stand that after we take the biopsy we close the perineum and await a permanent section in 48 hours, and then reopen the perineum and expose the prostate if the biopsy proves the lesion to be a carcinoma.

In conclusion, I would like to emphasize that the problem of benign prostatic hypertrophy is not a simple one and that there are many pitfalls and complications that can be encountered when a physician who is not specifically trained in urology attempts to handle the problem. It is likewise true that there is no more grateful patient than the man who has had difficulty urinating for many years and whose ability to urinate has been improved through treatment and surgery upon his prostate. It must be stressed again that every patient over the age of 50 who enters a physician's office should have a rectal examination to determine the size, consistency, and fixation of the prostate gland in order to rule out, or to identify as soon as possible, any areas of malignancy.

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AGE CHANGES IN COLOR MATCHING

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The present paper deals with age changes which occur in male and female subjects in color matching. Previous research on color vision and age has been meager and results have often been contradictory. Cook (3), studying color discrimination in children, found a significant increase in the accuracy of color discriminaton between the ages of 2 and 6 years but concluded that up to the age of 15 years children do not perform as well as adults on most color vision tests.

Smith (7) likewise reported that although infants are sensitive to color, children up to the age of 15 years are not as good as adults on color vision. He found a rapid increase in color discrimination to age 25 with no appreciable decline until after 65. However, he based his conclusions on a limited number of cases and did not report exact ages of subjects, so that the time when decline actually sets in could not be determined.

In the industrial field, Tiffin (8) made a study of 7,000 factory workers and reported progressive deterioration of ability to discriminate colors between the age of 25 and 55. He concluded that because of this decline, employees in industrial color work should have an initial excess of ability to discriminate colors.

More recently, Ouellette (5), in a pilot study of 28 male and female subjects between the ages of 20 and 30 years and 28 between 75 and 85 years, found significantly lower scores in the ability of the older group to match blue, red, green, and yellow. She found no significant sex differences but did find wide individual variations of color matching ability at all ages.

On the other hand, Boice, Tinker, and Paterson (1) studied 236 faculty members of the University of Minnesota on the Ishihara and American Optical Company color tests and found no deterioration between the ages of 20 and 59.

Chapanis (2) likewise found no significant relationship between age and color vision provided persons under 15 years of age were omitted. He gave 574 persons between the ages of 7 and 77 years 5 different tests at the Baltimore Sesquicentennial and based his conclusions on the results obtained. He found some statistically significant relationship between visual acuity and color vision but because some persons with poor visual acuity made better color scores than some with good visual acuity, he concluded that the relationship is insignificant from a practical point of view. Chapanis also reported in this article several studies which have tended to show that old people have greater difficulty than younger persons in distinguishing and recognizing blue-green, blue, and violet and pointed out that this is reasonable because of the yellowing of the lens with advancing years. This yellowing of the lens has the effect of a filter which makes the discrimination of a bluish color more difficult and may even lead to a form of tritanopia or blue-blindness.

Contradictory evidence for sex differences in color vision likewise exists. Early studies indicated a superiority of women over men, but Pickford (6) attributed this to the greater incidence of color blindness among men and to the greater familiarity of women with color names. Correcting for these defects, he found no sex differences in ability to discriminate colors.

As mentioned above, Ouellette (5) also failed to find sex differences among her two age groups in ability to match colors.

MATERIAL AND METHODS

In the present study 355 unselected subjects (160 male and 195 female) between the ages of 10 and 93 years were given the Color Aptitude Test designed by Dimmick and Foss (4) for the Inter-Society Color Council. Aside from Ouellette's (5) 28 institutionalized subjects and a few older men from the Sailor's Snug Harbor in Staten Island, all subjects were living in their own homes in the community. All took the test voluntarily and gave good cooperation. There were no noticeable variations in motivation at different age levels.

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The Color Aptitude Test consists of a flat rectangular board, 18 in. long and 14 in. wide, on which are mounted 4 rows of 12 plastic chips, (1″ × 1½″), each row containing fine graduations of shade of a particular hue—in order from top to bottom, blue, red, green, and yellow. The color series range in 15 regular and substantially equal saturation steps, with the exception of the least and the most saturated of each color which are farther apart in saturation than the other steps in the series. Since they are easier to match, these least and most saturated chips are placed in the end positions of each row on the chart and used by the subject as practice trials. The chips are mounted on the chart in such a way that all adjacent chips are at least three saturation steps apart.

A transparent plastic container holds 48 chips identical to those mounted on the board. They are arranged in the container in random order and are released one at a time by means of a plunger manipulated by the subject. The subject matches each chip from the container to one on the board, recording his choice on a paper score sheet which is placed behind the chips and so arranged that the number on back of the loose chip can be written directly beneath the chosen chip on the board. Each selection is recorded and the chip replaced in the plastic container before the next chip is drawn. Scores are determined from a scoring key which assigns a value of 3, 2, 1, or 0 for each selection according to the closeness of the match.

In the present study most of the subjects were given the test under daylight conditions. In so far as was possible, a bright day but without direct exposure to sunlight was chosen. However, a few took the test under four 100 watt daylight bulbs. Also, Ouellette's 28 subjects between 75 and 85 years of age were included, and these took the test under controlled light conditions—a 100 watt Macbeth lamp on each side of the board and a 200 watt Macbeth lamp at the top of the board in a darkened room. However, since comparison of these subjects with those who took the test under the less controlled daylight conditions showed practically identical scores for similar age groups, it is believed that the procedure is defensible for practical purposes.

TABLE 1. BARTLETT'S TEST OF HOMOGENEITY.

Group	Level of Significance of Heterogeneity
Sex	.63
Color	.25
Age	.70

RESULTS AND DISCUSSION

Starting at the age 10, subjects were grouped according to sex and age decade with the exception of the highest age group, which included individuals both in the eighties and the nineties.

Each individual was then scored for his performance on blue, red, green, and yellow and for his total performance. However, as the weighted scoring system does not yield the same total possible score for each color, raw scores were converted into percentages and are thus presented for the sake of uniformity.

Since perusal of the scores for each decade suggested differences between the groups, and since Bartlett's test of homogeneity showed the sex, color, and age groups to be homogeneous within themselves (see table 1), an analysis of variance was carried out in order to determine what proportion of the differences was due to age, sex, and color.

Analysis of variance: Table 2 reveals that the age groups show differences in scores which are reliable at the .01 level of confidence.

The colors likewise show score differences which are reliable at the .01 level of confidence.

Sex differences as a whole are reliable only at the .05 level of confidence, but interactions of sex and age and sex and color show differences reliable at the .01 level of confidence.

TABLE 2. ANALYSIS OF VARIANCE.

Source of Variance	Sum of Squares	Degrees of Freedom	Estimate of Variance	F Ratio	Level of Significance
Age	95,451	7	13 637	65.56	.01
Sex	1,302	1	1,302	6.25	.05
Tests	39,283	3	13,094	62.95	.01
Age × Sex	26,048	7	3,721	17.89	.01
Age × Tests	22,977	21	1,094	5.26	.01
Sex × Tests	4,712	3	1,571	7.55	.01
	189,773	42			
Total	476,694	1,419			
Residual	286,921	1,377	208		

Within the age groups, there are also significantly reliable differences (.01 level of confidence) between performances on the separate colors.

Color scores for sex and age groups: Table 3 shows the means and standard deviations of the male, female, and total individuals at each decade for blue, red, green, yellow, and total color score. As can be seen from this table, all scores, with the exception of yellow, which shows a slight rise in the forties, tend to show an initial rise from the 10 to 19 decade to reach a peak in the twenties and then to show a fairly steady decline to the oldest age group. Figure 1 depicts the age decline graphically and shows also more clearly the superiority at all ages of yellow and red over green and blue. It will be noted that the curves of decline are similar in form to those found on most other abilities studied. In order to learn something more about the sex, color, and age differences indicated by the analysis of variance and shown by the mean scores, the reliability of these differences was determined.

Table 4 shows the reliability of the sex differences found at each decade and for the total groups for each color and for total color score. From this it can be seen that the females are superior to the males in total color score at the .05 level of confidence. However, the females do not score significantly higher than the males on all colors nor at all decades of life. No one decade of life shows significant sex differences on any one color, although the total female group is superior to the total male group at the .01 level of confidence in ability to differentiate shades of red and at the .05 level of confidence in ability to differentiate shades of green. Also, the females attain higher total color scores than the males at the 10 to 19 decade (.05 level of confidence), at 40 decade (.02 level of confidence) and at the 70 decade

TABLE 3. SCORES FOR AGE, SEX, AND COLOR.

Age	Number	Blue		Red		Green		Yellow		Total Score	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
10-19	F 26	48.3	13.76	56.9	16.75	56.0	13.87	63.0	15.36	56.08	8.51
	M 20	49.3	16.29	48.5	11.45	50.6	12.81	54.0	13.33	50.21	7.87
	Total 46	48.8	15.19	53.2	15.57	53.7	13.94	59.1	15.47	53.53	8.70
20-29	F 26	59.0	11.25	57.8	14.94	54.1	15.55	57.4	14.14	57.20	10.28
	M 24	59.6	9.13	60.2	13.84	58.0	12.87	62.6	15.10	59.50	9.90
	Total 50	59.3	16.27	59.0	14.52	56.9	14.52	59.9	15.11	58.35	10.13
30-39	F 26	53.7	17.12	59.0	14.41	55.1	18.75	60.1	14.41	57.44	10.96
	M 19	50.8	15.80	54.8	16.04	51.7	16.27	57.5	13.80	53.19	10.84
	Total 45	52.4	16.35	57.2	15.16	53.6	17.74	59.0	14.15	55.65	11.05
40-49	F 26	48.8	18.28	60.7	13.20	55.4	16.38	62.7	12.25	56.87	10.05
	M 24	47.6	12.18	50.2	15.17	44.6	16.85	58.2	18.60	49.95	8.60
	Total 50	48.2	15.76	55.7	15.55	50.2	17.49	60.6	15.33	53.55	10.03
50-59	F 26	47.8	13.20	59.5	13.34	47.0	16.56	52.1	14.49	50.64	10.72
	M 24	43.8	17.66	50.9	13.47	45.3	13.97	56.3	15.04	49.07	10.42
	Total 50	45.9	15.49	55.4	14.11	46.1	15.46	54.1	14.95	49.89	10.35
60-69	F 21	35.4	18.64	52.7	22.68	39.5	16.61	48.1	16.42	44.07	10.42
	M 19	33.3	15.68	55.4	12.01	39.9	17.71	50.1	17.18	44.08	8.36
	Total 40	34.3	23.42	54.0	13.91	39.6	16.76	49.1	14.19	44.07	14.19
70-79	F 25	36.5	15.22	54.1	17.74	38.0	13.51	45.4	16.46	43.49	10.94
	M 11	31.2	12.52	36.4	20.42	21.1	12.43	35.7	12.60	31.10	7.23
	Total 36	34.9	14.83	48.7	20.47	32.8	15.80	42.4	16.13	39.70	11.57
80-89	F 19	29.8	15.33	37.6	14.91	26.2	13.00	35.0	14.55	32.12	10.08
	M 19	27.9	15.38	41.9	11.94	26.5	17.13	36.3	14.89	33.15	10.50
	Total 38	28.8	15.19	39.7	13.42	26.4	14.92	35.7	14.45	32.64	10.11
Total	F 195	45.74	16.81	55.47	16.12	47.9	16.82	53.8	16.72	50.56	12.78
	M 160	44.25	17.62	50.80	14.87	44.0	17.78	52.9	18.67	47.68	12.12
	Total 355	45.07	18.93	53.67	17.35	46.1	6.28	53.4	18.26		

(.01 level of confidence). No other significant sex differences are found. No explanation can be offered for the superiority of the female sex at some age levels and not at others, but it is possible that their superiority in matching reds and greens may be due to the greater incidence of undetected red-green color weakness among males.

Disregarding sex, table 5 shows the significance of the differences between the scores at the various age levels. From this it can be seen that the 10 decade is inferior to the 20 decade on all scores, and shows no significantly reliable superiority over the other age groups until the 50 decade is reached, when the superiority is significant at the .01 level of confidence and remains so throughout the remaining decades of life. In considering the separate colors, the 10 decade is superior to the 50 decade only in ability to differentiate shades of green (.02 level of confidence) but is superior to the 60 decade and beyond at the .01 level of confidence in ability to distinguish shades of blue, green, and yellow and superior only to the 80 decade in ability to differentiate shades of red. This is in keeping with the findings of Chapanis (2), Cook (3) and Smith (7) that up to age 15 children do not perform as well as adults on color tests. Although the reason for this is not certain, it seems likely that it may be due to a developmental factor. The 20 decade shows total color matching scores which are significantly better than those of all decades with the exception of the 30 decade. As for separate colors, the 20 decade is superior to the 30 decade only in ability to differentiate shades of blue (.05 level of confidence) but superior to all other decades (.01 level of confidence) in ability to differentiate shades of this color. On red, the twenties perform better than the tens but only at the .10 level of confidence and better than

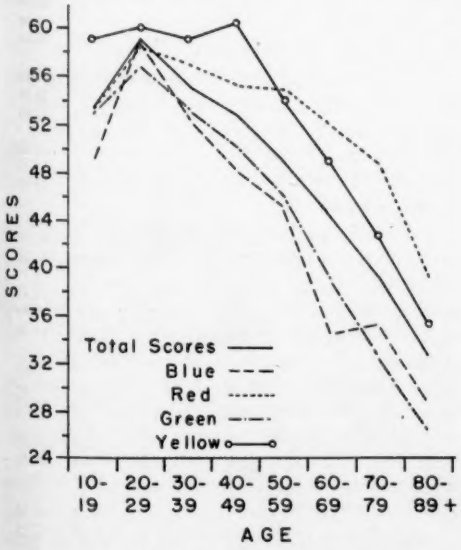

Fig. 1. Age changes in color matching scores.

TABLE 4. SIGNIFICANCE OF DIFFERENCES BETWEEN SCORES OF SEXES AT VARIOUS AGE LEVELS.

Levels of Confidence		Blue	Red	Green	Yellow	Total
10–19	F > M					.05
20–29						
30–39						
40–49						.02
50–59						
60–69						
70–79						.01
80 +						
Total			.01	.05		.05

TABLE 5. SIGNIFICANCE OF DIFFERENCES BETWEEN SCORES AT VARIOUS AGE LEVELS.

Levels of Confidence		Blue	Red	Green	Yellow	Total
10–19>	50–59			.02		.01
	60–69	.01		.01	.01	.01
	70–79	.01		.01	.01	.01
	80+	.01	.01	.01	.01	.01
20–29>	10	.01	.10			.02
	30	.05				
	40	.01		.10		.02
	50	.01		.01	.10	.01
	60, 70, 80	.01	.01	.01	.01	.01
30–39>	50	.05		.05		.02
	60	.01		.01	.02	.01
	70	.01	.05	.01	.01	.01
	80	.01	.01	.01	.01	.01
40–49>	50				.05	.10
	60	.01		.01	.01	.01
	70	.01	.10	.01	.01	.01
	80+	.01	.01	.01	.01	.01
50–59>	60	.01		.10		.05
	70	.01	.10	.01	.01	.01
	80+	.01	.01	.01	.01	.01
60–69>	70			.10	.10	
	80+		.01	.01	.01	.01
70–79>	80+	.10	.05	.10	.10	.01

the sixties and beyond at the .01 level of confidence. They perform better than the forties, but only at the .10 level, on green, but do better on this color at the .01 level at all decades from 50 on. Likewise, they perform better on yellow at all ages beyond sixty (.01 level) but only at the .10 level for the 50 year group.

The thirties attain significantly higher total color scores and scores in blue and green than all ages from 50 upwards. They are better than all from age 60 on in yellow and from age 70 on in red.

The forties are better than the fifties, but only at the .10 level of confidence, in total color score and at the .05 level of confidence in ability to differentiate shades of yellow. They are better, at the .01 level of confidence, than all ages from 60 upwards in total color scores and in ability to distinguish reds, greens, and yellows. Their superiority in red reaches only a .10 level of confidence at age 70 and a .01 at the eighties.

The fifties give better performance than those 60 and older on the color test as a whole and on the blues, and to a lesser extent (.10 level) on the greens but only attain reliably better scores than the seventies and eighties on red and yellow. The sixties do a little better than the seventies (.10 level of confidence) on the greens and yellows but show statistically reliable differences only when compared with the eighties in total score and in reds, greens, and yellows.

The seventies are better than those 80 and older on all color scores, although the differences in blue, green, and yellow are reliable only at the .10 level of confidence. Roughly speaking then, we might say that the results indicate that the ability to match colors develops in childhood to reach a peak in the twenties and from then on shows a gradual and steady decline to old age. Although the decline is steady, it is gradual so that the differences between adjacent decades are generally not statistically reliable; it is only when a decade intervenes that the results assume statistical reliability. Just what effect visual acuity has on these results is not known, but in view of the fact that practically all subjects of the 40 decade and beyond wear corrective lenses, it is reasonable to believe that there is some relationship between the two or at least that some common factor of aging is operating to lower efficiency in all visual functions. However, it must be noted that there were some individuals with known poor visual acuity who performed relatively well on the color test.

These findings do not support the contention of Ouellette (5) that no one color decreases in efficiency more rapidly than any other color. Rather, they are in accord with the findings reported by Chapanis (2) that there is a more rapid decline in ability to discriminate blue. Ability to discriminate shades of green likewise shows a relatively rapid decline with age, whereas the decline of ability to discriminate yellow and red (particularly the latter) seems to be less precipitous. To this writer also it seems reasonable to attribute this to the filtering effect of the progressive yellowing of the lens with advancing age. Practical implications of these findings for color workers in the industrial field might be made. In the first place, Tiffin's suggestion (8) that because of the decline of this ability with age, it would be desirable for workers in the color field to have an initial excess of ability to discriminate colors is a good one. Also in addition to the initial selection of efficient color matchers, it would be desirable to periodically check the efficiency of workers in this field, particularly those who work with blue and greens. It is not yet known definitely what part continued practice might play in the maintenance of efficiency of ability to match colors, but certainly the fact that the ability in general does decline with age should be considered.

Variability. No statistically reliable sex differences in variability are found at any age level or in any specific color, although there is some tendency (significant at the .10 level of confidence) for the men to be more variable than the women in their ability to match colors at ages 70 and 80.

There is a tendency for the 60 year old group to be more variable in their total scores on the color test than younger age groups. This greater variability is reliable at the .01 level of confidence when compared with those in the 10 to 19 year decade of life, at the .02 level when compared with 40 year olds, at the .05 level when compared with 20 and 50 year olds, and at the .10 level when compared with 30 year olds. The 70 year olds also show somewhat greater variability than the 10 year olds (.10 level of confidence).

A similar tendency is found for the 60 year olds to show greater variability in their ability

to differentiate shades of blue, this difference being reliable at the .05 level of confidence for the 10, 20, and 30 year groups, at the .02 level for the 40, 50, and 80 year groups, and at the .01 level for the 70 year group.

The 70 year group shows greater variability than other age groups in the differentiation of shades of red, this difference being reliable at the .05 level of confidence when compared with 20, 50, 60, and 80 year olds and at the .10 level when compared with 10, 30, and 40 year olds.

There are no other statistically reliable differences in variability between age groups although it must be noted that all groups show wide individual variation in ability to match colors whether this ability be considered in general or in regard to specific colors.

SUMMARY

In order to determine age changes which occur in male and female subjects in color matching, 355 unselected subjects (180 males and 195 females) between the ages of 10 and 93 were given the Color Aptitude Test. Subjects were grouped according to sex and decade of life, with the exception of the 80 and 90 year olds, who were treated as one group, and their mean total color scores and mean scores on blue, red, green, and yellow compared.

Results showed a rise in separate and total color scores from the first decade (10 to 19 year group) to the twenties with a subsequent steady decline.

Shades of blue and green proved to be more difficult to discriminate between than shades of yellow and red at all ages, and the ability to discriminate between these shades likewise showed a more rapid decline with age.

At all ages, but particularly in the sixties, wide individual differences were found in ability to match colors. Females showed an inconsistent tendency to score better than males at certain decades of life and also on certain colors.

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THE ATTITUDES OF SPECIAL GROUPS TOWARD THE EMPLOYMENT OF OLDER PERSONS

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One factor of importance in the field of aging is the attitude held by various groups who deal with older workers toward the actual employment of such workers. Yet a review of the vast array of psychologic literature related to aging indicates that little can be found on this specific topic. For example, Tuckman and Lorge (5, 6, 7, 8) have analyzed attitudes of graduate and under-graduate students and retired workers, both institutionalized and non-institutionalized, toward older workers. Thomas and Osborne (4) have interviewed employers in England about their favorableness toward older employees, while the Bureau of National Affairs (1) has reported the opinion of their employer panel on certain aspects of older employee's performance.

In general, however, a large gap exists in the specific problem of attitudes toward employment of older persons, particularly those held by persons who presumably are nearest these older persons. Probably the only attempt to measure directly these attitudes toward employment by means of an attitude scale has been made by Kirchner, Lindbom, and Paterson (3). In this study, a specific scale was developed and applied in many situations.

The present paper constitutes an extension of this past work. Responses of such varied groups as personnel people and supervisory trainees are shown.

METHOD

The 24 item attitude scale, measuring attitudes toward employment of older persons, which was developed in earlier studies (3) was given to 7 different groups over a two-year period, either by mail or by group testing. Basic information for the various samples is given below.

1) *Old Age Psychologists:* This group was made up of 93 psychologists from Division 20, Maturity and Old Age, of the American Psychological Association. Presumably these were people with a major interest in gerontology, and they were selected for that reason.

2) *Experimental Psychologists:* Sample size for this group of psychologists from Division 3, Experimental, of the American Psychological Association was 82. This group was postulated to have little interest in problems of older employees and was selected as a contrast to other groups of psychologists.

3) *Minnesota Psychologists:* This group represents a more general section of psychologists with interests in all fields of psychology. All 195 were members of the Minnesota Psychological Association.

4) *Vocational Guidance Workers:* These 98 people were members of the Minneapolis Vocational Guidance Association. Their basic interest lay in guidance work, where many or all had occasion to counsel older workers and job applicants.

5) *Supervisory Trainees:* Supervisors or near supervisors enrolled in night extension courses in effective supervision made up this sample (N = 120). These persons either supervised older persons directly or worked side-by-side with them.

6) *Nationwide Personnel:* A random sample (N = 180) of personnel men and women throughout the U. S. was selected from a comprehensive list developed by the Industrial Relations Center at the University of Minnesota. Personnel people who deal directly in selection of employees would have close contacts with older employees.

7) *Twin City Personnel:* A local group of personnel people were selected from the Minneapolis - St. Paul area (N = 85). Selection of this group was for the same reason as the national group, and also added local color to the study.

Six of the 7 groups were surveyed by mail; one group (supervisory trainees) was tested directly. Of the mail surveys, the percentage of responses, including follow-ups, was as follows;

Old Age Psychologists	92%
Experimental Psychologists	83%
Minnesota Psychologists	91%
Vocational Guidance Workers	60%
Nationwide Personnel	71%
Twin City Personnel	82%

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TABLE 1. RESPONSES OF SEVEN GROUPS TO 24 ITEMS MEASURING ATTITUDES TOWARD EMPLOYMENT OF OLDER PERSONS (PERCENTAGE).

1. I think older employees have fewer accidents on the job.

	SA	A	U	D	SD*
O. A. Psy.	18	44	34	3	0
Exper. Psy.	6	43	41	10	0
Minn. Psy.	5	55	28	13	0
Voc. Guid.	6	50	31	13	0
Supervis.	10	58	14	18	0
Nat. Pers.	8	63	9	18	1
T. C. Pers.	1	57	20	22	0

2. Most companies are unfair to older employees.

	SA	A	U	D	SD
O. A. Psy.	8	44	24	24	1
Exper. Psy.	2	30	44	21	2
Minn. Psy.	6	23	28	41	2
Voc. Guid.	3	23	28	46	0
Supervis.	2	17	17	55	8
Nat. Pers.	2	6	11	64	17
T. C. Pers.	4	6	16	60	14

3. Older employees are harder to train for jobs.

	SA	A	U	D	SD
O. A. Psy.	3	43	19	32	1
Exper. Psy.	4	30	28	36	4
Minn. Psy.	1	48	15	33	3
Voc. Guid.	1	34	18	40	3
Supervis.	7	45	12	33	2
Nat. Pers.	3	45	14	36	2
T. C. Pers.	2	46	20	27	5

4. Older employees are absent more often than younger employees (under age 30).

	SA	A	U	D	SD
O. A. Psy.	0	5	13	54	27
Exper. Psy.	0	14	35	45	15
Minn. Psy.	0	3	14	59	24
Voc. Guid.	0	5	8	63	23
Supervis.	2	21	19	50	8
Nat. Pers.	4	18	15	57	4
T. C. Pers.	4	21	10	62	4

5. Younger people (under age 30) act too smart nowadays.

	SA	A	U	D	SD
O. A. Psy.	3	14	12	54	17
Exper. Psy.	0	10	10	51	21
Minn. Psy.	2	8	10	67	13
Voc. Guid.	3	8	8	66	13
Supervis.	2	21	19	50	8
Nat. Pers.	4	18	15	57	4
T. C. Pers.	4	21	10	62	4

6. Younger employees (under 30) usually have more serious accidents than older employees.

	SA	A	U	D	SD
O. A. Psy.	3	39	40	24	4
Exper. Psy.	1	17	68	12	1
Minn. Psy.	3	30	44	21	2
Voc. Guid.	2	30	39	27	3
Supervis.	2	44	23	28	2
Nat. Pers.	1	41	22	32	2
T. C. Pers.	4	22	37	33	4

7. In a case where two people can do a job about the same, I'd pick the older person for the job.

	SA	A	U	D	SD
O. A. Psy.	3	34	37	25	1
Exper. Psy.	2	16	50	32	0
Minn. Psy.	2	27	44	26	1
Voc. Guid.	2	20	47	27	2
Supervis.	6	26	29	34	5
Nat. Pers.	1	39	27	31	1
T. C. Pers.	1	26	31	38	3

8. I think that Social Security payments are too small.

	SA	A	U	D	SD
O. A. Psy.	20	49	13	8	1
Exper. Psy.	24	43	22	7	4
Minn. Psy.	25	43	17	14	2
Voc. Guid.	14	46	18	16	3
Supervis.	12	31	14	32	9
Nat. Pers.	12	36	16	31	4
T. C. Pers.	12	37	9	40	3

9. Occupational diseases are more likely to occur among younger employees (those under age 30).

	SA	A	U	D	SD
O. A. Psy.	1	6	32	49	11
Exper. Psy.	1	6	44	32	17
Minn. Psy.	0	9	27	53	10
Voc. Guid.	0	9	30	50	10
Supervis.	1	13	23	51	10
Nat. Pers.	0	15	22	54	9
T. C. Pers.	0	10	35	42	14

10. The older employees usually turn out work of higher quality.

	SA	A	U	D	SD
O. A. Psy.	2	47	41	10	0
Exper. Psy.	4	22	54	20	1
Minn. Psy.	3	40	37	20	0
Voc. Guid.	3	47	21	28	0
Supervis.	6	56	11	26	0
Nat. Pers.	5	56	13	24	1
T. C. Pers.	1	48	19	31	1

11. I think that older employees are more grouchy on the job.

	SA	A	U	D	SD
O. A. Psy.	0	17	33	48	1
Exper. Psy.	1	11	38	41	6
Minn. Psy.	1	16	18	59	5
Voc. Guid.	0	17	15	62	5
Supervis.	5	28	15	46	5
Nat. Pers.	1	21	15	60	4
T. C. Pers.	0	21	15	60	4

12. I believe that older people cooperate more on the job.

	SA	A	U	D	SD
O. A. Psy.	1	12	48	9	0
Exper. Psy.	0	29	46	24	1
Minn. Psy.	4	40	34	22	0
Voc. Guid.	1	45	35	19	0
Supervis.	4	45	17	35	0
Nat. Pers.	2	61	18	19	0
T. C. Pers.	1	46	26	26	1

13. Older people seem to be happier on the job.

	SA	A	U	D	SD
O. A. Psy.	0	37	48	14	1
Exper. Psy.	0	26	57	17	0
Minn. Psy.	4	38	39	19	0
Voc. Guid.	3	36	37	24	0
Supervis.	7	55	13	26	0
Nat. Pers.	3	57	19	21	1
T. C. Pers.	6	43	21	30	0

14. I feel that older people are more dependable.

	SA	A	U	D	SD
O. A. Psy.	15	65	15	5	0
Exper. Psy.	6	55	30	9	0
Minn. Psy.	9	61	21	8	1
Voc. Guid.	5	62	16	16	0
Supervis.	12	66	7	16	0
Nat. Pers.	11	71	12	6	1
T. C. Pers.	6	67	10	17	0

15. Most older people cannot keep up with the speed needed in modern industry.

	SA	A	U	D	SD
O. A. Psy.	2	25	26	45	2
Exper. Psy.	2	17	44	33	4
Minn. Psy.	1	19	18	56	5
Voc. Guid.	1	18	18	58	4
Supervis.	5	24	9	57	3
Nat. Pers.	1	26	15	55	2
T. C. Pers.	1	22	20	54	1

16. Supervisors find it hard to get older people to adopt new methods on the job.

	SA	A	U	D	SD
O. A. Psy.	1	19	28	51	1
Exper. Psy.	1	41	48	9	0
Minn. Psy.	2	48	23	26	1
Voc. Guid.	1	40	18	40	1
Supervis.	9	49	15	23	2
Nat. Pers.	4	53	10	31	1
T. C. Pers.	5	65	14	16	0

17. Older people should get higher wages for their jobs.

	SA	A	U	D	SD
O. A. Psy.	1	9	29	53	9
Exper. Psy.	1	17	33	43	6
Minn. Psy.	2	12	23	55	8
Voc. Guid.	0	6	17	66	10
Supervis.	2	10	11	62	15
Nat. Pers.	0	3	6	73	18
T. C. Pers.	1	7	12	63	16

18. You'll find that the employees who are most loyal to the company are the older employees.

	SA	A	U	D	SD
O. A. Psy.	12	48	29	10	1
Exper. Psy.	1	46	43	9	0
Minn. Psy.	8	48	27	15	1
Voc. Guid.	5	49	23	21	1
Supervis.	11	50	8	27	2
Nat. Pers.	9	59	14	17	0
T. C. Pers.	10	46	17	25	2

19. Older people are too set in their ways—they don't want to change.

	SA	A	U	D	SD
O. A. Psy.	0	37	27	34	2
Exper. Psy.	2	37	39	30	1
Minn. Psy.	1	25	28	44	2
Voc. Guid.	0	22	22	52	3
Supervis.	8	44	13	31	2
Nat. Pers.	2	45	11	41	1
T. C. Pers.	4	43	17	35	1

20. I think older employees have as much ability to learn new methods as other employees.

	SA	A	U	D	SD
O. A. Psy.	5	47	17	28	1
Exper. Psy.	9	32	22	37	1
Minn. Psy.	4	52	12	31	1
Voc. Guid.	3	67	7	21	1
Supervis.	2	63	7	22	4
Nat. Pers.	1	59	11	29	1
T. C. Pers.	2	54	12	30	1

21. I think companies should train middle-aged employees (those aged 35-50) to handle many different jobs.

	SA	A	U	D	SD
O. A. Psy.	13	51	30	6	0
Exper. Psy.	12	45	30	10	1
Minn. Psy.	12	54	24	9	1
Voc. Guid.	9	60	23	7	0
Supervis.	7	52	17	20	2
Nat. Pers.	10	61	16	12	1
T. C. Pers.	9	56	20	16	0

22. I think that older employees make better employees.

	SA	A	U	D	SD
O. A. Psy.	5	32	54	9	0
Exper. Psy.	0	18	62	20	0
Minn. Psy.	4	38	39	18	0
Voc. Guid.	1	34	40	24	0
Supervis.	2	38	19	36	2
Nat. Pers.	2	44	23	29	1
T. C. Pers.	1	25	48	26	0

23. I think that most younger people are too radical in their ideas.

	SA	A	U	D	SD
O. A. Psy.	3	4	8	61	24
Exper. Psy.	0	0	15	54	32
Minn. Psy.	3	2	5	69	21
Voc. Guid.	0	7	7	62	23
Supervis.	0	12	16	61	10
Nat. Pers.	1	8	16	69	6
T. C. Pers.	1	9	19	59	11

24. Pay should be based on length of service rather than on what a person does (how long a person has worked in a company should count more than the amount of work he turns out).

	SA	A	U	D	SD
O. A. Psy.	0	1	13	60	26
Exper. Psy.	0	5	20	54	22
Minn. Psy.	1	5	13	55	26
Voc. Guid.	0	4	8	56	32
Supervis.	0	2	5	51	40
Nat. Pers.	1	0	2	46	51
T. C. Pers.	1	1	1	37	59

* SA = Strongly Agree; A = Agree; U = Undecided; D = Disagree; SD = Strongly Disagree.

No significant differences in responses were found between replies to the first mailing and replies to follow-ups in these samples. All replies within each group were summarized as a whole. The total number of usable replies for all 6 groups was 853.

RESULTS AND DISCUSSION

Table 1 shows the distribution of responses made on each item to each of 5 possible choices ranging from Strongly Agree to Strongly Disagree. The following facts seem apparent.

1. Psychologists, as represented by the 3 psychologist groups and the vocational guidance workers, are much more undecided in their response than the 3 non-psychologist groups. On every item except numbers 5 and 23, the psychologists have a greater percentage of undecided responses. The statistical significance of such differences is shown in table 2. Of the 22 items that showed greater undecided responses for psychologists, 17 of these differences are significant at the 5% level of confidence: One hypothesis for these results is that psychologists, by and large, may be more sophisticated as far as attitude questionnaries go and thus tend to avoid agreeing or disagreeing with generalized statements. Items 1, 10, 12, and 13 in particular show large differences in the percentage of undecided responses used for psychologists and non-psychologists.

2. Both groups, psychologists and non-psychologists, tend to avoid the extreme responses, Strongly Agree or Strongly Disagree. On only 10 of the 24 items, can percentage figures of 10 per cent or greater be found for these extremes; figures of 20 per cent and above can be found on only 5 of the 24 items. The greatest use of an extreme response is found on item 24 (Pay should be based on length of service rather than on what a person does). There is a high degree of use of the category, Strongly Disagree (from 22 per cent to 59 per cent). One probable interpretation is that none of these attitudes as reflected by the items are held very fervently and that the ego-involvement of the persons sampled in these problems is not great.

3. Over-all analysis of each item is a difficult matter. There is general agreement that older persons have fewer accidents on the job (item 1), cooperate more on the job (item 12), are more dependable (item 14), and are most loyal to the company (item 18). On the other hand, there is also general agreement that older employees are harder to train for jobs (item 3), cannot keep up with the speed needed in modern industry (item 15), and should not get higher wages for their jobs (item 17). Items, however, show wide variation in percentage responses (see item 16). Certainly the concept of individual differences is shown well.

4. Responses of old age psychologists do not differ very greatly from those of other psychologists. There is a tendency, however, for their replies to be more favorable toward employment of older persons than those of other groups.

5. The sample of experimental psychologists uses the undecided response much more than the other samples. On 20 of the 24 items, experimental psychologists favor the undecided response more than the other participating groups. This probably reflects a don't know or don't care attitude rather than a true neutral position on each item. For example, Dunnette, Uphoff, and Aylward (2) have shown that lack of information significantly increases the use of the undecided response on a union scale. This group, in a sense, is somewhat set aside from the problems of old age and should be lacking in information and/or interest about aging.

6. The biggest difference between psychologists and non-psychologists occurs on item 2 (Most companies are unfair to older employees). The percentage of the responses in each category for both large groups is shown below:

	SA	A	U	D	SD*
Psychologists	5	29	30	35	1
Non-psychologists	3	10	14	60	13

The differences between both groups on Agree, Undecided, Disagree, and Strongly Disagree responses are statistically significant. There is a suspicion that personnel people believe there is a direct reference to themselves in this item and hence are strongly defensive. On other items there is no suggestion of either great antagonism or great liking for older employees. In many cases non-psychologists are more favorable toward the older employees than the old age psychologists (i.e., item 20—I think older employees have as much ability to learn new methods as other employees). Likewise they are apt to be more unfavorable (i.e., item 16—Supervisors find it hard to get other persons to adopt new methods on the job). No consistent pattern emerges.

TABLE 2. AVERAGE DISTRIBUTION OF "UNDECIDED" RESPONSES FOR PSYCHOLOGISTS AND NON-PSYCHOLOGISTS FOR THE 24 ATTITUDE ITEMS (PERCENTAGE).

Item No.	Psychologists	Non-psychologists	Difference	Significance* at 5% Level
1	32	13	19	Yes
2	30	14	16	Yes
3	19	15	4	No
4	16	15	1	No
5	10	15	— 5	No
6	46	26	20	Yes
7	44	28	16	Yes
8	17	14	3	No
9	30	25	5	No
10	37	14	23	Yes
11	24	16	8	Yes
12	39	19	20	Yes
13	44	18	26	Yes
14	20	10	10	Yes
15	24	14	10	Yes
16	27	12	15	Yes
17	25	9	16	Yes
18	29	13	16	Yes
19	27	13	14	Yes
20	14	10	4	No
21	26	17	9	Yes
22	46	27	19	Yes
23	8	17	— 9	Yes
24	13	3	10	Yes

* Significance of differences was determined by means of Zubin's tables (9).

SUMMARY

Responses of 7 different groups (N = 853) to each of 24 attitude items measuring the at-

* For explanation of the abbreviations, see footnote to table 1.

titude toward employment of older persons have been shown. All groups tend to avoid extreme responses; psychologists use the undecided response to a much larger degree than non-psychologists. Psychologists who have stated interests in old age problems tend to be more favorable in attitude than other groups. Psychologists whose basic interest is in experimental psychology are less extreme in responses and somewhat more unfavorable in attitude. Psychologists who have varying interests seem to be more favorable. However, differences are not great and vary widely from item to item. For non-psychologists, replies are roughly neutral.

Over-all there seems to be no great like or dislike for employment of older persons, which may reflect the fact that the items are not particularly ego-involved for any group. In one instance where an item seems directly ego-involved to a group, there is a strong feeling on the question.

The results may stem also from the large amount of psychologic training that most of these respondents have received with the presumed stress on recognition of individual differences.

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Book Reviews

FIVE HUNDRED OVER SIXTY, a Community Survey of Aging, by Bernard Kutner and David Fanshel, Alice M. Togo, and Thomas S. Langner. Russell Sage Foundation, New York, 1956, 345 pages, $4.00.

In many respects this is the best of the growing number of so-called community surveys of older people to emerge from the rapidly developing interest in this age group. Actually the report is a partial—yet comprehensive and detailed—analysis of extensive questionnaire data gathered from a stratified sample of 500 men and women aged 60 years and above drawn from a population comprised of more than a quarter of a million people living in the 409 block area served by the Kips Bay-Yorkville Health Center of New York City. This district of Manhattan contains in addition to spectacular buildings a closely packed population exceedingly varied in socio-economic condition and ethnic origin.

A compelling desire to know better how to serve the tremendous number of elderly people in this section prompted the study. The interdisciplinary research team organized for this purpose directed its attention toward four specific problems:

(1) personal adjustment,
(2) factors affecting or affected by health,
(3) the use of community health services,
(4) attitudes toward health and social centers.

The object of the research was to uncover information useful in guiding the development of service programs for older people. The establishment of the Adult Counseling Center by the Health Center as a direct outgrowth of the study concretely demonstrates the value of the investigation.

The authors do much to clarify the complex interrelationships between various motivational factors which affect the utilization of public and private health and other services available to older people in the community. An important contribution of the study is the reasonable demonstration that no one type of health service will have universal appeal to older people. Thus the controversial question of the advisability of age specific geriatric clinics is answered by pointing out that they are highly acceptable to some and rejected by others. To be effective health services must be attractive to oldsters. This in turn requires that attention be paid to the multiple motivational factors which influence use and acceptance of the service.

The study raises many questions and suggests many answers with varying degrees of success. Future workers in the field should go over this report carefully so as to plan their research better to answer questions unanswerable by the data of the present study. Thus future surveys carried on within a well defined conceptual framework should produce a systematically increasing body of knowledge upon which can be built powerful action programs directed toward the amelioration of age associated problems.

ROBERT W. KLEEMEIER, Ph.D., Director,
The Moosehaven Research Laboratory
Orange Park, Florida

PSYCHOLOGICAL ASPECTS OF AGING, PROCEEDINGS OF A CONFERENCE ON PLANNING RESEARCH, John E. Anderson, Editor, American Psychological Association, Washington, D. C., 1956, 323 pages, $2.00.

This well-edited book contains a uniquely broad, but integrated, survey of gerontologic research and presents a wealth of excellent proposals and hypotheses for future research. It is particularly this latter feature which makes this volume purposeful and timely.

The conference was organized into five symposia with representatives from many disciplines:

1. "Assessment of adjustment to the environment" with discussions by Robert Havighurst, Jack Weinberg, Raymond Kuhlen, William E. Henry, and Robert Peck.
2. "Genetic, environmental, psychological, and biological methods" with papers by John E. Anderson, John B. Calhoun, Franz Kallmann, Eugene Streicher, James Birren, and Robert Kleemeier.
3. "Perception and Intellect" presented by Harold E. Jones, Ivan Mensch, Oscar Kaplan, Nancy Bayley, and William A. Owens.
4. "Learning, Motivation and Education" outlined by Herbert Conrad, Martin Loeb, Wayne Dennis, Sidney Pressey, Wilma Donahue, and Irving Lorge.
5. "Functional Efficiency, Skills and Employment" by Ross McFarland, Milton Barron, Charles Odell, Josef Brožek, and Nathan Shock.

Each section was introduced and later integrated by one person. These are worth while and move beyond the usual banal role often taken by discussants who merely reiterate what has occurred. Perhaps the over-all high quality of this volume reflects not only the effects of getting a group of active and competent research workers together, but also is the result of careful planning which seemingly gave each contributor a focussed role for his participation. The planning committee consisted of James Birren, Robert Havighurst, Harold E. Jones, and John E. Anderson.

In addition to the individual papers, three appended sections will be of value to those in or contemplating research in aging. There is a summary of the conferences by the editor, John E. Anderson, who gave his presentation a novel twist by covering past, current, and proposed research at an equivalent grammatical tense, giving the reader an over-all view without the distraction of shifts in tense between literature references, work currently in progress, and ideas which emerged in the conference discussions. There follows a survey of current aging research by James Birren, a set of rating scales for assessing social adjustment in adult and later maturity by Robert Havighurst, and an extensive reference list compiled from the papers of the entire volume. The conference was opened by an address by J. H. Sheldon, of England, the retiring President of the International Association of Gerontology, who viewed the social and mental life of the aged person by analyzing

his own cogent and strikingly clever clinical observations on the problems of the decline in postural skill in old age.

This report contains many ideas that can be of use to those contemplating research or engaged in care and management problems and is especially stimulating because of the varied approaches and disciplines of the participants.

MARGARET THALER, Ph.D.
Walter Reed Army Institute of Research
Washington, D. C.

ORGANIZED HOME MEDICAL CARE IN NEW YORK CITY, A Study of 19 Programs by the Hospital Council of Greater New York, Published for the Commonwealth Fund by Harvard University Press, Cambridge, Massachusetts, 1956, 538 pages, $8.00.

Nearly 49 per cent of the more than 2,000 persons who at the time of this Study received organized home medical care in New York City were 65 years of age and older. The periods of care at home averaged well over one year. These few figures may suffice to show the significance of organized home care in medical programs for the aged and to indicate the interest that this first comprehensive review and evaluation of home care programs has for the readers of the Journal of Gerontology.

Conducted by the Hospital Council of Greater New York, a coordinating and planning agency, the Study goes, both in the analysis of present and in the recommendations for future programs, far beyond the description of such services; it extends into over-all community planning by defining the role of home care in integrated medical care programs.

The *ad hoc* appointed Study staff successfully combined the skills of a physician—Dr. Peter Rogatz—and a sociologist—Guido Crocetti—, technically advised by a statistical consultant, Dr. John E. Silson. Most of the wealth of information that the book provides in its text and in numerous tables was secured by consultation of members of the professional team of each of the 19 programs and by interviews of a generous sample of patients and their families. The recommendations, sound and useful since based on evaluation of, and conclusions from, present experiences, cover a wide area, including such administrative measures as staff composition, qualification of individual staff members, methods of referral and evaluation of patients, and record keeping. This reviewer considered as most significant one of the basic recommendations which stipulates that home medical care should be operated within the context of total medical care, preferably in association with a general hospital. The comprehensive, extremely carefully prepared bibliography, calls for particular commendation.

Although the Study deals, primarily, with home care services in New York City, the findings definitely are of general interest and the recommendations apply to communities anywhere. It is fortunate indeed that this thorough and detailed Study becomes available at a time when large numbers of agencies and communities have to cope with the problem of adequate medical care programs for the increasing number of aged people with chronic diseases. The Greater New York Hospital Council's timely publication will be of substantial help and guidance to many of them.

MARTA FRAENKEL, M.D.
New York City Department of Hospitals

BOOKS RECEIVED

Care of the Long-Term Patient, Chronic Illness in the United States, Volume II. Harvard University Press, 1956, 606 pages, $8.50.

Economic Status of the Aged, by Peter O. Steiner and Robert Dorfman, University of California Press, Berkeley, 1957, 296 pages, $5.00.

Flexible Retirement, Evolving Policies and Programs for Industry and Labor, edited by Geneva Mathiasen, G. P. Putnam's Sons, New York, 1957, 226 pages.

New Jersey Old Age Study Commission, a Positive Policy Toward Aging, Senator Walter H. Jones, Chairman, 1957, 217 pages, paper bound.

Organized Home Medical Care in New York City, a Study of Nineteen Programs by the Hospital Council of Greater New York, Harvard University Press, 1956, 538 pages, $8.00 (reviewed in this issue, page 222).

Retirement, a New Way of Life, a Guide to the Later Years, by Eugene Friedmann, University of Wisconsin Extension Division Publication, 1956, 41 pages, paper bound, 50 cents.

Studies of the Aged and Aging, Selected Documents. Vol. V, Public and Private Services for Older People: Rehabilitation, Housing and Living Arrangements, Education, and Community Services, Nov., 1956, 161 pages. *Vol. VI, Care of the Aging by the Veterans' Administration,* Nov., 1956, 92 pages. *Vol. VII, Guide to Significant Publications,* Nov., 1956, 242 pages. *Vol. IX, Research, Demonstration and Training,* Dec., 1956, 157 pages. Compiled by the staff of the Committee on Labor and Public Welfare, United States Senate, U. S. Government Printing Office, Washington, D. C.

Summary of Federal Legislation Relating to Older Persons, Dec., 1956, 34 pages, prepared by the Committee on Labor and Public Welfare, United States Senate, by the Legislative Reference Service, Library of Congress, U. S. Government Printing Office, Washington, D. C.

Toward Good Housing for the Aged, selected articles from the Journal of Housing, NAHRO reprints, 1956, National Association of Housing and Redevelopment Officials, 1313 East 60 St., Chicago 37, Ill., 40 pages, paper bound, $1.00.

JOURNAL OF GERONTOLOGY

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Section C

Organization Section
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Reports from Foreign Correspondents

ITALIAN CONTRIBUTIONS TO GERONTOLOGY

GIORGIO MARS

Research and investigations in the domain of gerontology started officially in Italy in 1950 and went on continuously, increasing especially in the last three years, relative to the ever-increasing number of elderly people in the population in general and in the institutions for care of the sick in particular.

At the beginning of 1954 a National Meeting of Gerontology was held in Rome, and on that occasion Antonietti and Scopinaro (Genova) examined in detail the problems relating to endocrinology in old age, while Daddi (Milan) summarized the views of the authoritative schools of phthisiology in Italy about the clinical and sociologic aspects of senile tuberculosis.

Later on, the Lombard Section of the Italian Society of Gerontology was founded in Milan with Professor Melli (medical pathologist of Milan), as its president.

A delegation presided over by Professor Greppi (medical clinician of Florence) carried the contributions of the Italian Schools to the 3rd International Congress on Gerontology in London, illustrating especially the metabolic aspects of senile pathology; while the participation of famous foreign clinicians gave particular importance to the Symposium on Senescence of the Osteoarticular System, which was held in Salsomaggiore.

In 1955 the Ministry of Public Education approved the institution of a lectureship on Gerontology and Geriatrics at the University of Florence, and Professor Greppi, President of the Italian Gerontological Society, was entrusted with the new charge.

The 5th National Meeting on Gerontology was held in Turin, before which reports were read from Bastai (biological fundaments of senescence), Favilli and Manganotti (senescence of the cutaneous system), Malan (surgery in the elderly), De Gironcoli (prostate and senility), and Pellegrini (insurance and senility).

Among the many other manifestations, it is worth while mentioning the foundation of the Emilian Section of the Italian Society of Gerontology, whose chair was taken by Sotgiu (medical clinician of Bologna). The "Geriatric Week" saw at Verona scientists from all parts of Italy, who discussed problems concerning geriatrics, with special regard to some senile dysendocrinisms and to physiocrenotherapy of the affections in senescence.

In 1956 the Italian Parliament and Senate ratified the extension of sanitary and social assistance to the elderly and retired, which provision represents at least a partial solution to the complex and delicate problems of geriatric assistance.

Sotgiu's report (Bologna) on physiopathology of the digestive system in old age and Patrassi's report (Padua) on physiopathology of the liver in advanced age formed the fulcrum of the Geriatric Days held at Montecatini. Then at Florence a course of lectures was delivered by the most distinguished lecturers of the local University for the purpose of reporting the latest advances in the fields of gerontology and geriatrics.

During the meeting of the Lombard Section of the Italian Gerontological Society the following subjects were discussed: the aspecific antihyaluronidase principle of serum in elderly people (Mars and Castellani), senile diabetes (Greppi), and quinidine therapy of atrial myocardial-coronarosclerotic fibrillation (Morpurgo and Mars).

Finally at the 6th National Meeting held in Naples, Pietrantoni (Milan) and Arslan (Padua) discussed conditions of the auditory system in the elderly, Coppo (Modena) the vitamin therapy in advanced age, and Patrono (Rome) hormone therapy in senescence and senility. There was also a special participant honoring the meeting, Stepp, from the Medical Clinic of München, who illustrated the relationship between nutrition and aging.

Italy is now preparing to receive the 4th International Congress of Gerontology that will be held at Merano and Venice in July, 1957, in which the most distinguished scientists from all over the world have by now announced their participation. At Merano different subjects such as the various aspects of senescence of cardiovascular, respiratory, and endocrine systems will be discussed, besides the problems of the rehabilitation of chronic patients and many other topics. At Venice the Congress will devote particular attention to the medical and social aspects of nervous diseases in aging.

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INDEX TO CURRENT PERIODICAL LITERATURE

NATHAN W. SHOCK
Gerontology Branch
National Heart Institute
National Institutes of Health

THE SUBJECT categories are those in *A Classified Bibliography of Gerontology and Geriatrics* by Nathan W. Shock published by Stanford University Press, Stanford, California, (1951). Only major headings are used and the Roman numerals correspond to those given in the bibliography. In so far as possible, references are classified according to organ system. Thus, most of the material on Geriatrics will be found under the organ system involved in the disease. Cross references are indicated by number at the end of each section. When available, abstract references are given *(B.A.—Biological Abstracts: P.A.—Psychological Abstracts, and P.I.—Population Index)*. Abbreviations for journals are those used in *A World List of Scientific Periodicals Published in the years 1900-1933*, 2nd Edition. For journals not listed, abbreviations were devised according to the general rules used in the above source. It is impossible to cover all journals and list all papers concerned with aging and the aged. Authors and publishers are requested to call attention to publications or to send reprints to the Gerontology Branch, Baltimore City Hospitals, Baltimore 24, Maryland. The assistance of the Forest Park Foundation, Peoria, Illinois, in the preparation of these materials is gratefully acknowledged.

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